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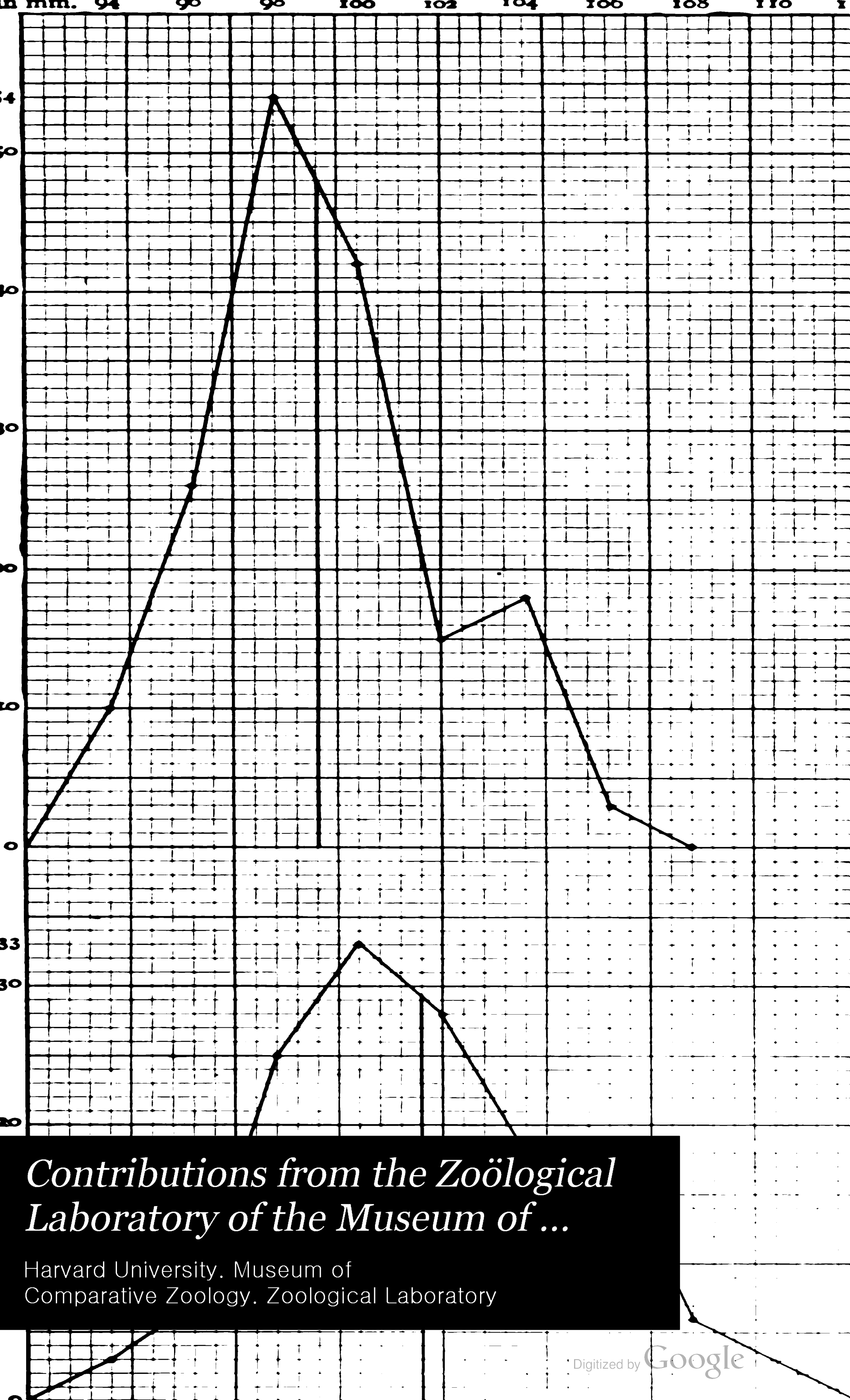

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE
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COLLEGE. E. L. MARK, DIRECTOR.

No. 121.

A QUANTITATIVE STUDY OF VARIATION IN THE SMALLER NORTH-AMERICAN SHRIKES.

BY R. M. STRONG.

WITH EIGHT FIGURES.

REPRINTED FROM THE AMERICAN NATURALIST,
VOL. XXXV., No. 412.

CAMBRIDGE, MASS., U. S. A.

APRIL, 1901.

FEB 26 1902

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R. M. STRONG.

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I. INTRODUCTION.

THE work described in this paper was begun in the fall of 1898, and was carried on at Harvard University during the year 1898–99 under the direction of Dr. Charles B. Davenport. It was completed during the winter of 1899–1900.

It was my desire to employ statistical methods in the study of variation in a group of birds, and to apply the "Precise

Criterion of Species" of Davenport ('98) to a problem in bird classification. The smaller American shrikes of the genus Lanius appeared to me to offer favorable material for the application of quantitative methods to the solution of an interesting taxonomic problem.

The shrikes are a group of passerine birds more or less generally distributed in northern Europe and North America, and probably of circumboreal origin. The large northern shrike, *Lanius borealis* Vieill., of North America grades into the great gray shrike, *L. excubitor* Linn., of Europe; and it exhibits a strong tendency towards individual variation.

In the United States, Mexico, and southern Canada the breeding[1] shrikes are not essentially different from the northern shrike, *L. borealis*, in certain of its color phases, except for smaller size and the more or less complete disappearance of a conspicuous barring or mottling of the breast in adults. This barring of the breast is persistent in most adults of *L. borealis* and characteristic of the juvenile plumage of the southern shrikes, a fact of much phylogenetic interest.

According to the nomenclature of the American Ornithologists' Union there are at present recognized three races or subspecies of the southern or smaller shrikes, which are as follows: *Lanius ludovicianus ludovicianus* Linn., *L. ludovicianus excubitorides* Swains., and *L. ludovicianus gambelli* Ridgw. Subspecies *gambelli* includes the shrikes of California and vicinity. The shrikes of the rest of the country are classed as either ludovicianus or excubitorides, the former being typical in the south central states and the latter in the vicinity of Colorado.

The shrikes of New England and the north central states have been variously classed, by different systematists, as either ludovicianus or excubitorides. Palmer ('98) has proposed for the shrikes of this intermediate region a new subspecies, migrans, whose validity, I believe, can be well tested by the "Precise Criterion" method.

---

[1] In this paper the breeding range only is considered in discussing geographical distribution.

We have in the shrikes the following eight important variable characters:

1. Length of tail.
2. Length of wing.
3. Length of bill.
4. Depth of bill.
5. Curvature of culmen.
6. Color of dorsal surface of head and back.
7. Color of upper tail coverts.
8. Color of breast.

II. Material.

While the shrikes make good subjects for a study of variation, there has been an unfortunate difficulty in obtaining material in amounts as large as is desirable. Shrikes are not especially common birds in the regions most collected in, and they are considered rare in most parts of New England. They are certainly not abundantly represented in collections of birds, and many of the skins that were obtained were imperfect, or did not come from the breeding areas of the birds.

I have been able to procure measurements of two hundred and ninety-four available skins, obtained from the following sources:

Mr. William Brewster, Cambridge, Mass.	174 skins.
National Museum, Washington, D. C.	64 "
Museum of Comparative Zoölogy	18 "
Mr. J. H. Gaut, Washington, D. C.	15 "
Mr. C. F. Batchelder, Cambridge, Mass.	8 "
Dr. A. K. Fisher, Washington, D. C.	5 "
Mr. N. Hollister, Delavan, Wis.	4 "
Oberlin College Museum, Oberlin, Ohio	4 "
Carnegie Museum, Pittsburg, Pa.	1 "
Mr. William Palmer, Washington, D. C.	1 "

The numbers given do not include skins which, for various reasons, could not be used. I wish here to express my thanks to the gentlemen who have secured for me the loan of the above-mentioned material, and especially to Mr. William Brewster for many courtesies received.

GEOGRAPHICAL DISTRIBUTION.

	MALE.	FEMALE.		MALE.	FEMALE.
Arizona	16	13	Montana	4	3
British Columbia	1	0	Nevada	2	0
California	15	21	New Mexico	0	1
Colorado	1	2	New York	5	2
Connecticut	1	0	North Carolina	3	0
District of Columbia	6	2	North Dakota	1	0
Florida	45	23	Ohio	3	1
Georgia	8	6	Ontario	0	1
Illinois	10	3	Pennsylvania	1	1
Indiana	1	0	South Carolina	16	2
Indian Territory	0	1	Tennessee	1	1
Kentucky	1	0	Texas	7	5
Maine	0	1	Utah	0	1
Massachusetts	1	2	Virginia	4	10
Mexico	17	16	Wisconsin	0	1
Minnesota	1	0	Wyoming	3	1
			Total	174	120

III. METHODS.

A. General Precautions.

It is hardly necessary to say that work of this kind should be done with material in normal condition. I have made no measurements where mutilations existed, and only adult birds bearing good evidence of representing their breeding ranges have been used. It has been necessary to reject a number of skins because of lack of data as to sex. As male and female shrikes are essentially alike in color and differ little in size, it is not possible to verify the original determinations of sex; however, there has been no noticeable confusion in this respect.

No attention has been given to the subspecific distinctions appearing on the labels of skins, for I have considered it important to avoid all possibility of bias of opinion which attention to previous classification might give.

B. Measurements of Variable Characters.

1. *Linear Measurements. Length of Wing.* — The shortest distance between the wrist and the tip of the wing was measured with a pair of dividers, the wing being folded naturally at the side of the body. The left wing was taken in every case for the measurement.

Length of Tail. — This measurement was made from the papilla of the uropygial gland to the extreme end of the tail feathers. One arm of the dividers was placed with its point resting on the anterior face of the papilla. On account of mutilation and frequent difficulty in finding the papilla, the measurement has often to be omitted. Great precision cannot be obtained, as there is irregularity in the relative position of the papilla in the drying of the skin, and there is also usually some wearing away of the distal ends of the rectrices. When the latter were much frayed no measurement was attempted.

Length of Bill. — The only satisfactory method found for measuring the length of the bill was to take the shortest distance between the nostril and the distal apex of the upper mandible. The point of one arm of the dividers was placed against the most distal face of the nostril. I have had to make allowance in some cases for the wearing away of the distal apex of the upper mandible, though the amount of wear is too small to materially affect the measurement.

Depth of Bill. — The greatest dorso-ventral diameter of the bill near its base was determined. There is considerable liability to error here on account of lack of uniformity in the articulation of the mandibles in the dried skin, and I found it necessary to reject quite a number of skins on this account.

2. *Curvature of Bill.* — This is a character of the distal half of the culmen. Its quantitative expression is a matter of some difficulty, for we have here a curve which is not the arc of a circle nor a parabola, nor does it correspond to any geometrical figure. Then, too, the form and size of the bill are such as to render it impracticable to make precise measurements directly. To meet the latter difficulty it was decided to try to trace on paper the enlarged projection of the outline of the bill. After

some experimentation, the following apparatus was devised. Fig. 1 is a diagram of an enlarging camera with a box in front of the lens, at the left in the figure. A circular aperture for the passage of light was made in one side of the box, the opposite side being removed. At one side of the aperture (*A*, Fig. 2) a simple cylinder (*C*, Figs. 1 and 2) of stiff paper just large enough to admit a shrike skin was fastened to the outside of the box in a nearly horizontal position by a single tack.

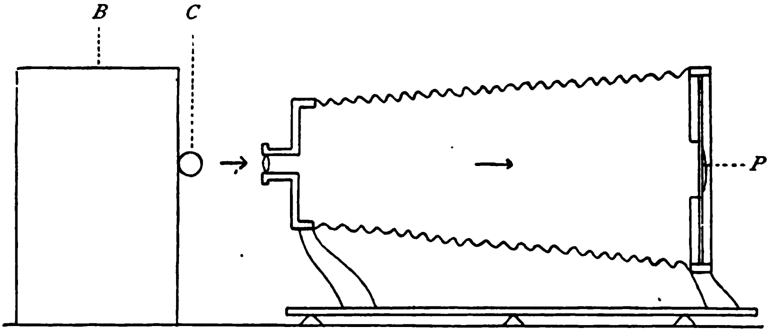

FIG. 1. — Longitudinal section of enlarging camera apparatus. *B*, box; *C*, paper cylinder; *P*, photographic paper in holder.

Orientation was then secured for any skin by the following simple adjustments: (1) rotation of the skin in the cylinder around its longitudinal axis; (2) rotation of the cylinder on the axis formed by the tack; (3) moving the skin in the cylinder towards or away from the aperture; (4) rotation of the box on a vertical axis.

The apparatus was arranged so that the bill of a shrike was about seven inches from the front of the lens and its median plane at right angles to the axis of the camera. Strong diffused daylight was then allowed to pass through the aperture of the box past the bill, and into the lens of the camera, as shown by the arrows in Fig. 1. In the plateholder at the back of the camera was placed a piece of "velox" paper (a rapid printing photographic paper) about 2×3 inches

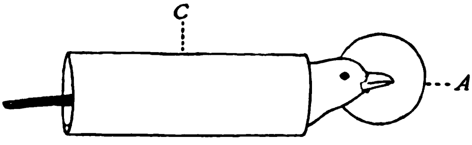

FIG. 2. — Paper cylinder (*C*) holding bird so that the bill projects before aperture (*A*).

in size. An exposure of seventy-five seconds was ordinarily sufficient to obtain a picture, which appeared on development as a white area on a black ground. A magnification of 3⅓ diameters was secured, care always being taken to have the distance of the bill from the lens constant. An outline of the culmen of practicable working size having been thus obtained, the next step was the analysis of the curve.

It was highly desirable to have one simple criterion of the curvature, if one sufficiently representative could be found. In Fig. 3, which represents the outline in a representative case, a great increase in the sharpness of the curvature is seen towards the distal end of the culmen; the sharpness of curvature varies in different individuals. The point where the rapid increase begins was found on inspection to be sufficiently uniform in position to suggest the idea of comparing in different individuals the angle embraced between two chords of the curve of the culmen, each of them terminating at the distal end of the culmen. The proximal ends of the two chords were selected by the following method: one was established at a point on the culmen as far from its apex as the apex was from the nearest margin of the nostril. The distance of the nostril from the tip of the culmen, of course, could not be measured on the photographic silhouette print; it was therefore determined

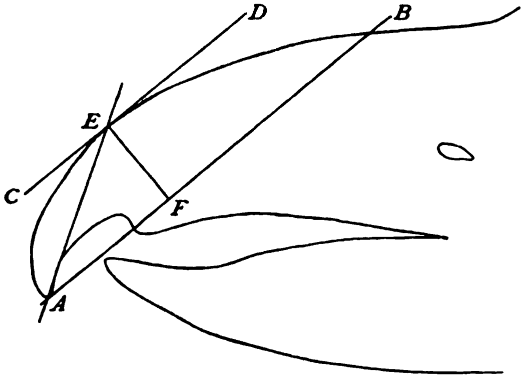

FIG. 3. — Diagram of bill showing method of measuring curvature of culmen. *AB* equals distance of *A* from nearest margin of nostril; *CD*, tangent to outline of culmen parallel to *AB*; *EF*, perpendicular at point of tangency, *E*; *EAF*, angle embraced between the two chords of the culmen, *AB* and *AE*.

by measuring the actual distance in the specimen and multiplying that value by the linear magnification of the print. The nostril distance was used because the nostril was found to be the only point sufficiently definite and constant for the proximal limit. This chord (*AB*, Fig. 3) served as a base line. A line (*CD*) parallel to the base line and tangent to the culmen was next drawn, and at the point of tangency (*E*) a perpendicular (*EF*) was erected between the lines. It was then a simple matter to measure the lines *AF* and *EF*, and with a table of natural tangents to determine the angle *EAF*. This is the angle that has been adopted as a criterion of curvature in the shrike bill. It is easily seen that there might be an infinite number of small variations in the outline of the culmen, especially in the part distal to the point *E*, which would not affect the angle *EAF*; but I believe that a correlation is to be expected between the curvatures in various portions of the culmen, so that it is probable that any considerable variation in outline elsewhere would affect the curvature in the region of *E* and thus find expression in the angle *EAF*. In order, however, to have something more than a subjective impression on this matter, I made a second series of measurements with half the length of the line *AB* used as a chord. The result of these measurements, and their deviations from those of the first set, are given on page 294.

3. *Color Measurements.*—For the quantitative determination of color, the "color mixer" was suggested. A simple form of color mixer — the Bradley Milton Color-Top — was employed. This instrument has a graduated disk, with superimposed paper disks of five different colors, which can be so adjusted as to make compound disks exposing two or more colors. On being rotated at high speed these colors are mixed into one, which is a combination of the colors used in the proportions of the number of degrees of each exposed. With the aid of this apparatus it is therefore possible to determine the proportions of these primary colors entering into any color effect which may be produced by various combinations of them. To determine the composition of any color in nature, it is necessary to reproduce empirically that color in the top and note the

combinations that have given the desired effect. In practice, however, the following conditions were found essential to accurate estimation of color: (1) Uniform strong diffused daylight, preferably coming from a skylight or north window and not subject to strong reflections from colored surfaces; (2) frequent comparison with previously made estimates; (3) careful comparison of the color obtained by the top with that of the plumage, whose color is being measured, both being viewed from different directions.

I found it impracticable to make color estimates for more than two hours continuously, because of color fatigue of the eye.

In spite of the above precautions, there are limitations to the use of the color-top. In the estimation of color, the personal equation plays an important part. Then, too, the surface of the color disks is very different in character from that of feathers. The peculiar luster or sheen so characteristic of a bird's plumage cannot be imitated by the color mixer. In estimating the color of the breast, more or less mottling, which exists in some cases, increased the difficulty. In such cases I have attempted to estimate the mean color. Some apparatus for blending a complex pattern into one color would be very desirable. I do not attempt to maintain that estimates of the same material by another person would exactly agree with mine; but I believe that I have a consistent classification of individuals according to color.

After several weeks of experimentation I found that, though there were traces of blue, red, and yellow, the colors black and white were by far the most important elements in the areas measured. Therefore I have taken as the color criterion the amount of black, or the melanism, of the color area described; and color estimates appear in the tables as percentages of melanism. I have found it more difficult to estimate colors containing more than 50% of white than those in which black predominated, and I have not attempted to make fine distinctions as to melanism in the lighter color areas. The large amount of black necessary to produce even the lightest grays in the color mixer was a matter of surprise to me. To properly appreciate the estimates of color given in this paper, a color mixer should be used to reproduce the combinations given.

Ability in the discrimination of color I have found to be much developed by experience. The color determinations of my first three months' work were rejected, as increased power of discrimination made more accurate results possible.

C. Geographical Areas.

The material described in this paper has been collected from a territory of great size and varying conditions, and it is therefore desirable to compare individuals both as parts of a single group and in subdivisions corresponding to natural life areas, so that correlations between individual variability and geographical variation may be made. I have adopted the life areas employed by Allen ('93) for the territory covered by my material, which are four in number:

1. *Austroriparian Subprovince*, embracing North and South Carolina, Georgia, and Florida.
2. *Appalachian Subprovince*, embracing Maine, Massachusetts, Connecticut, District of Columbia, Virginia, Ontario, New York, Pennsylvania, Indiana, Kentucky, Ohio, Illinois, Wisconsin, Minnesota, and North Dakota.
3. *Campestrian Subprovince*, embracing British Columbia, Montana, Wyoming, Colorado, Indian Territory, New Mexico, Texas, Idaho, Utah, Arizona, and Nevada.
4. *Sonoran Subprovince*, embracing California, Lower California, and Mexico.

A comparison of variations for still smaller areas is desirable, but this, to be useful, would require more material than I have been able to obtain. The subspecies of shrikes have the distribution given by Palmer ('98), excepting gambelli (Palmer did not consider the western shrikes), which agrees very well with the above life areas. The inhabitants of each are:

SUBPROVINCE.	SUBSPECIES.
Austroriparian	= ludovicianus
Appalachian	= migrans
Campestrian	= excubitorides
[Sonoran	= gambelli]?

IV. Results.

A. Indices of Variability.

1. *Wing and Tail.* — In Figs. 4–8, frequency polygons based on measurements of various dimensions, the position of the mean class is indicated by a heavy vertical line. In Fig. 4 are given frequency polygons for the lengths of the wing (4 *A*) and tail (4 *B*) of all available male shrikes. They show a striking absence of variability for these characters in a series of individuals representing four subprovinces with greatly varying conditions.

In Tables I–III are given correlations between the length of wing and the length of tail. I have used the method of Duncker (Davenport, '99, p. 33) in determining coefficients of correlation. The "probable error" has also been determined by the following formula (Davenport, '99, p. 34), in which ρ is the coefficient of correlation.

$$\text{P. E.}\ \rho = \frac{0.6745\,(1 - \rho^2)}{\sqrt{n}\,(1 + \rho)}$$

On comparing Tables II and III, we find that Florida shrikes have a greater length of tail in relation to the length of wing than shrikes from the Appalachian subprovince.

The correlation in these characters for the series from the Appalachian subprovince is seen to be 0.157− greater than that of the Florida series. This difference is possibly due in part to the skewness of the curves of frequency for these series, which renders precise correlations difficult.

Table I. — 140 Male Shrikes from the Entire Region.

Mean of wing = 99.06 + mm. Mean of tail = 101.571 + mm.
σ_1 = 2.74 + mm. σ_2 = 3.48 + mm.

		Wing, Subject.				Tail, Relative.					
X_2		−7	−5	−3	−1	0	2	4	6	8	
Rel. class		94	96	98	100	102	104	106	108	110	mm.
Sub. class (I)											(II)
	X_1										
94 mm.	−5			4	3	1					
96 mm.	−3	2	2	6	8	3		1			
98 mm.	−1	1	3	9	11	11	6	4			
100 mm.	0		1	5	9	6	7	6	2		
102 mm.	2			2	1	6	3	2			
104 mm.	4					1		5	3	3	
106 mm. (III)	6						2		1		(IV.)

$\rho = 0.5688 +$ P. E. $\rho = \pm\ 0.038 +$

TABLE II. — 41 MALE SHRIKES FROM FLORIDA.

Mean of wing = 97.463 + mm. Mean of tail = 102.292 + mm.
σ_1 = 2.29 + mm. σ_2 = 3.14 + mm.

		WING, SUBJECT.				TAIL, RELATIVE.				
X_2		−6	−4	−2	0	1	3	5	7	
Rel. class . . .		96	98	100	102	104	106	108	110	mm.
Sub. class (I) .										(II)
X_1										
94 mm.	−3 .		4	3	1					
96 mm.	−1 .	1		5	1					
98 mm.	0 .		1	1	7	4	3			
100 mm.	2 .				2	2	3	2		
102 mm.	4 .									
104 mm. (III)	6 .								1	(IV)

$\rho = 0.639 +$ P. E. $\rho = \pm 0.0487$

TABLE III. — 28 MALE SHRIKES FROM THE APPALACHIAN SUBPROVINCE.

Mean of wing = 99.357 + mm. Mean of tail = 99.43 + mm.
σ_1 = 2.31 + mm. σ_2 = 3.063 + mm.

		WING, SUBJECT.			TAIL, RELATIVE.				
X_2	. . .	−5	−3	−1	0	2	4	6	
Rel. class	. .	94	96	98	100	102	104	106	mm.
Sub. class (I)	. .								(II)
X_1									
96 mm.	−3 . .	2		1	1				
98 mm.	−1 . .	1	1	5	4				
100 mm.	0 . .			2	4				
102 mm.	2 . .			1		2	1	1	
104 mm.	4 . .							1	
106 mm. (III)	6 . .						1		(IV)

$\rho = 0.796 +$ P. E. $\rho = \pm 0.0348 +$

A general tendency towards great length of tail in southern birds has been noted by Allen ('71, pp. 230, 231). In Table VII, p. 291, the mode of lengths of tail for Austroriparian males is seen to be 102 mm.; whereas Appalachian males have a mode of 100 mm. The mean of Austroriparian shrikes is 101.91 + mm., while that of Appalachian shrikes is 99.43 + mm.,

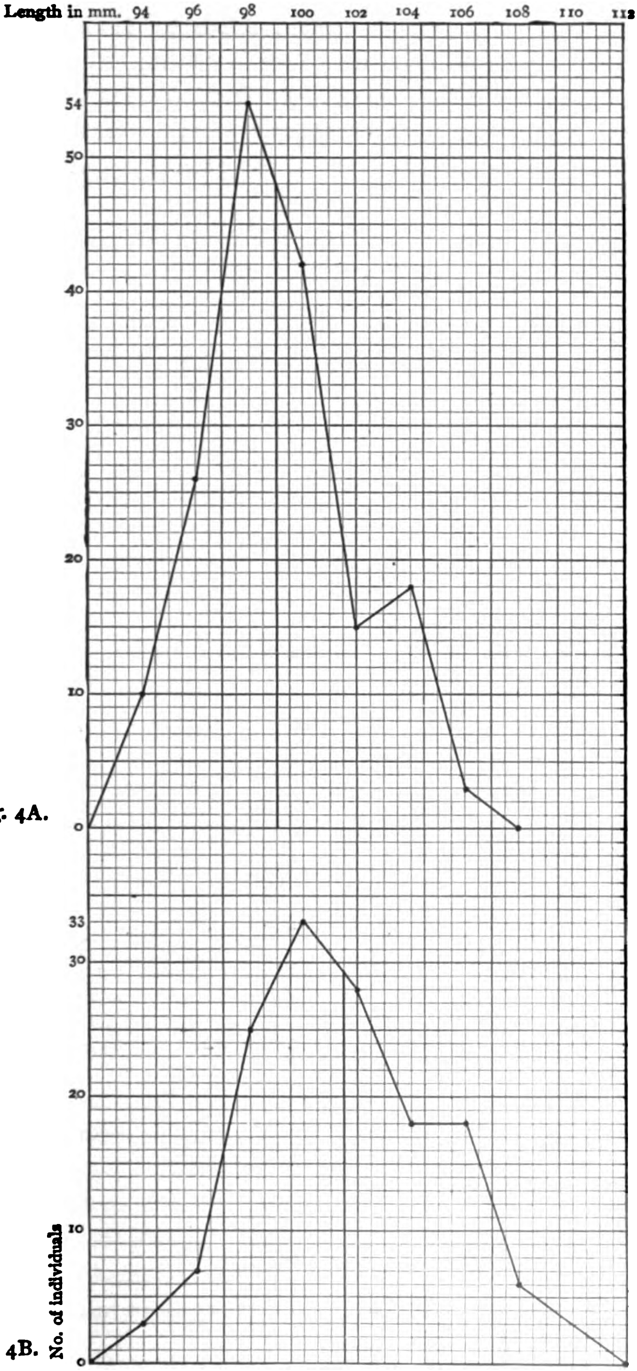

Fig. 4A.

Fig. 4B.

Fig. 4.—Frequency polygons. *A*, Classes of left wings for 168 males from entire region; *B*, tails of 141 males from entire region.

a difference of 2.48 mm. Austroriparian females show a still greater preponderance in length of tail, having a mode 6 mm. greater and a mean 3.05 mm. greater. Not much importance, however, can be attached to the great difference of the modes in this case, because of the small number of females measured.

Palmer ('98) says, in referring to shrikes of the Appalachian and Austroriparian subprovinces respectively: "In migrans [Appalachian] the wing is longer than the tail, due to its migratory habit; in ludovicianus [Austroriparian] the tail is longest, thus indicating its fixed habitat."

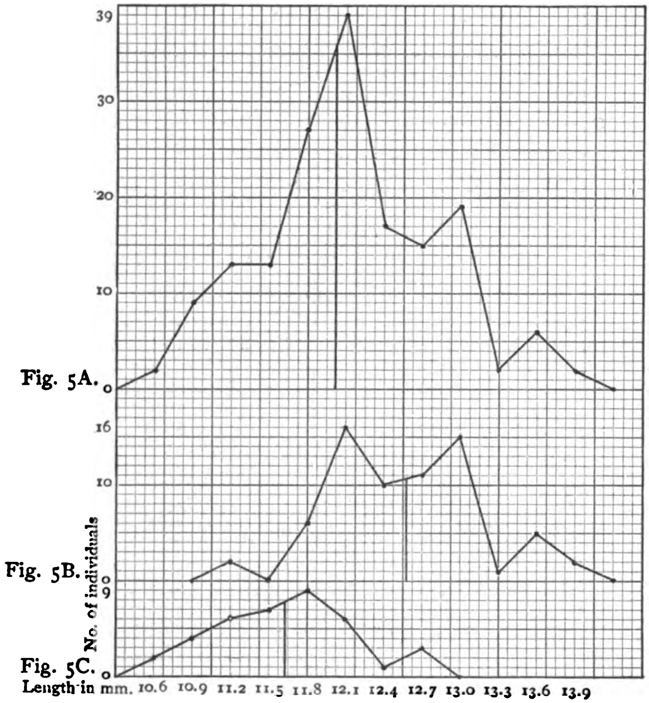

FIG. 5. — Frequency polygons for length of bill. *A*, 164 males from entire region; *B*, 69 males from Austroriparian subprovince; *C*, 38 males from Appalachian subprovince.

I find, on the contrary, that the wing is not absolutely longer than the tail in the Appalachian form, though from Tables VI and VII, pp. 290, 291, it is clear that the excess of the length of tail over that of the wing is less in migrans than in ludovicianus;

or, in other words, that *relatively to the tail,* the wing is longer in migrans than in ludovicianus, as his theory would require.

Palmer's explanation of length of wing in migrans as the result of habit is plausible. However, it has not been proved that shrikes which migrate northward actually fly any more than those remaining in Florida; moreover, it seems to me that there are other possible explanations. I am inclined to think that we have here a case of the condition already noted by Allen — a tendency towards greater length of tail in tropical birds. Unfortunately I cannot prove this, — any more than Palmer does his hypothesis, — for it has not been possible to determine the actual ratios of tail length and wing length with reference to the general size of the bird.

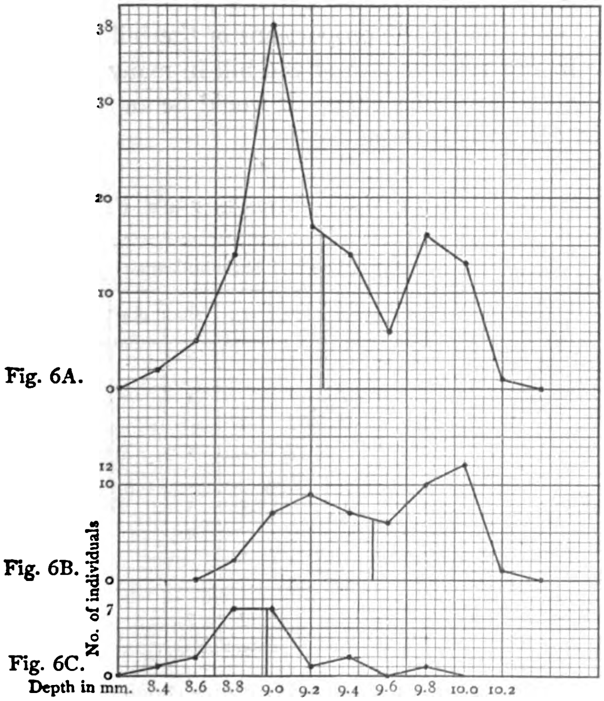

FIG. 6. — Frequency polygons for depth of bill. *A*, 126 males from entire region; *B*, 54 males from Austroriparian subprovince; *C*, 21 males from Appalachian subprovince.

2. *Length and Depth of Bill.* — In Fig. 5 are given frequency polygons of variations in length of bill for the entire region and

for two subprovinces. Here is shown a considerable separation of the means (indicated by the vertical line) for the subprovince polygons, indicating a larger bill for Austroriparian shrikes (5*B*), which are also seen in Fig. 6*B* to have a greater depth of bill. The increase in size found in the bills of Florida shrikes is also shown by comparison of Tables IV and V, in which these characters are correlated for a series of Florida[1] shrikes and a series from the Appalachian subprovince. A striking absence of correlation and a great tendency towards individual variation in the proportions of the bill are to be seen. Increase in the relative size of the bill in many southern birds has been noted by Allen ('71). It would be an interesting field for statistical investigation.

TABLE IV. — 32 MALE SHRIKES FROM FLORIDA.

Classes of Depth of Bill.	Classes of Length of Bill in Millimeters.										Total.
	11.2	11.5	11.8	12.1	12.4	12.7	13.0	13.3	13.6	13.9	
8.8 mm. . .				1	1						2
9.0 mm. . .				1			1				2
9.2 mm. . .				2		1			1	1	5
9.4 mm. . .			1				1		1		3
9.6 mm. . .					2						2
9.8 mm. . .			1	1		1	1		2		6
10.0 mm. . .			1	5	1		4				11
10.2 mm. . .						1					1
Total . . .			3	10	4	3	7		4	1	32

3. *Color.* — Frequency polygons for variations in the melanism of the head are shown in Fig. 7. The upper polygon (Fig. 7*A*), which includes the entire series, is seen to be distinctly bimodal, and in the lower polygons we see that the mode of greater melanism is due to the presence of Austroriparian shrikes, and that the shrikes of the Campestrian subprovince have the least melanism for the dorsal surface of the head.

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[1] Only Florida representatives of the Austroriparian shrikes were taken in this case, in order to eliminate intermediate forms, for it seemed desirable to me to compare the Appalachian shrikes with a group as nearly tropical as possible.

The Campestrian subprovince includes arid portions of the United States, where paleness of color in the fauna is supposed to be correlated with this condition.

Table V. — 22 Male Shrikes from the Appalachian Subprovince.

Classes of Depth of Bill.	Classes of Length of Bill in Millimeters.									Total.
	10.6	10.9	11.2	11.5	11.8	12.1	12.4	12.7	13.	
8.4 mm.			1							1
8.6 mm.	1								1	2
8.8 mm.			1	3		1				5
9.0 mm.	1	1	2	1	1	1		1		8
9.2 mm.			1			1				2
9.4 mm.				1		1	1			3
9.6 mm.										0
9.8 mm.			1							1
Total.	2	1	6	5	1	4	1	1	1	22

While such correlations are frequently suggested by the fauna of desert regions, the relations of humidity to color are still problems for further investigation.

Very great variations in the melanism of the upper tail coverts are shown in Fig. 8. The shrikes of the Campestrian subprovince have here also a mode of little melanism, 30%, while a mode of great melanism, 70%, is found for Austroriparian shrikes. The upper tail coverts of Campestrian shrikes, especially those from Colorado and Arizona, are very light gray. This peculiarity has caused the race to be designated as the white-rumped shrikes.

B. Tables of Constants of Frequency Polygons.

In Tables VI–XIII will be found indices of variation for all the material studied. All computations have been made from measurements grouped into classes. This grouping has been adopted both in drawing the polygons and in determining the indices of variability, in order to reduce the "probable error."

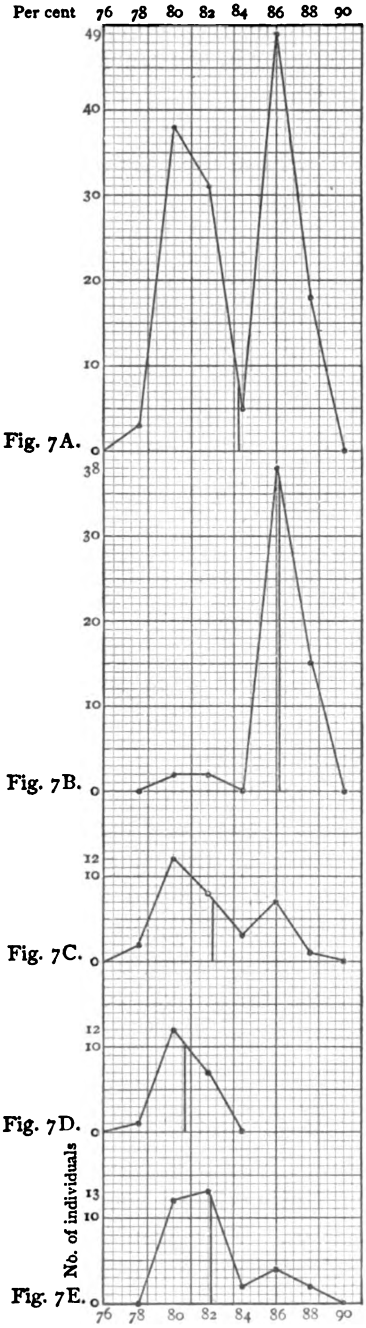
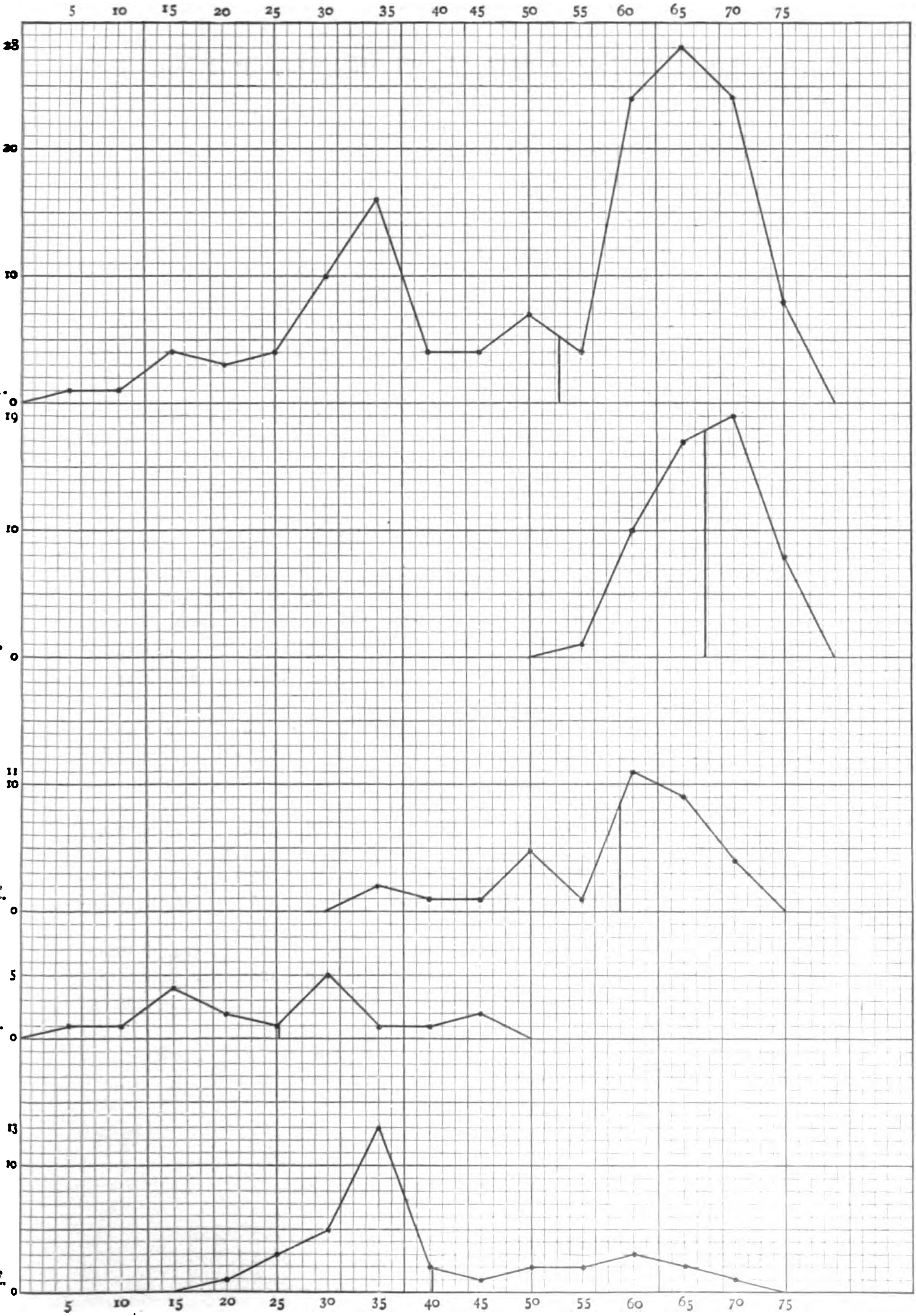


FIG. 7. — Frequency polygons for melanism of top of head. *A*, 144 males from entire region; *B*, 57 males from Austroriparian subprovince; *C*, 33 males from Appalachian subprovince; *D*, 21 males from Campestrian subprovince; *E*, 33 males from Sonoran subprovince.



—Frequency polygons for melanism of upper tail coverts. *A*, 142 males from entire region: *B*, 55 males from Austroriparian subprovince; *C*, 34 males from Appalachian subprovince; *D*, 18 males from Campestrian subprovince; *E*, 35 males from Sonoran subprovince.

The following constants of the frequency polygons have been determined (Davenport, '99):

1. Mode: Class of greatest frequency.
2. Mean: $M. = \frac{\Sigma(V.f)}{n}$.
3. Standard Deviation, $\sigma = \sqrt{\frac{\Sigma(x.^2f)}{n}}$.
4. Coefficient of variability: $CV. = \frac{\sigma}{M} \times 100$.
5. Probable error of Mean: $P.\ E.\ M.$[sub.] $= \pm 0.6745 \frac{\sigma}{\sqrt{n}}$.
6. Probable error of Standard Deviation: $P.\ E.\ \sigma$[sub.] $= \pm 0.6745 \frac{\sigma}{\sqrt{2n}}$.

TABLE VI. — LENGTH OF LEFT WING.

AREA.	MATERIAL.	MODE.	MEAN.	STANDARD DEV.
Entire region	168 males.		99.09 + mm.	2.79 + mm.
	112 females.		97.98 + mm.	2.64 + mm.
Austroriparian	70 males.	98 mm.	97.6 + mm.	2.04 + mm.
	31 females.	96 mm.	96.64 + mm.	2.35 + mm.
Appalachian	35 males.	98–100 mm.	99.2 mm.	2.45 + mm.
	25 females.	98 mm.	97.68 mm.	2.17 + mm.
Campestrian	27 males.	104 mm.	101.26 mm.	2.45 + mm.
	24 females.	102 mm.	99.75 mm.	2.79 + mm.
Sonoran	36 males.	98 mm.	100.28 mm.	2.90 + mm.
	32 females.	98 mm.	98.19 + mm.	2.36 + mm.

CV. of 168 males = 2.81 +. *CV.* of 112 females = 2.69 +.

P. E. M. [sub.] 168 males = ± 0.145.

P. E. σ [sub.] 168 males = ± 0.1026 +.

TABLE VII. — LENGTH OF TAIL.

AREA.	MATERIAL.	MODE.	MEAN.	STANDARD DEV.
Entire region .	141 males.		101.55 + mm.	3.49 + mm.
	95 females.		99.55 + mm.	3.63 + mm.
Austroriparian .	65 males.	102 mm.	101.91 + mm.	3.12 + mm.
	30 females.	104 mm.	100.47 + mm.	3.25 + mm.
Appalachian . .	28 males.	100 mm.	99.43 + mm.	3.06 + mm.
	19 females.	98 mm.	97.42 + mm.	3.85 + mm.
Campestrian . .	20 males.	104 mm.	102.3 mm.	3.48 + mm.
	18 females.	102 mm.	99.89 + mm.	3.42 + mm.
Sonoran . . .	28 males.	100 mm.	102.21 + mm.	3.90 + mm.
	28 females.	102 mm.	99.43 + mm.	3.88 + mm.

C V. of 141 males = 3.43 +. *C V.* of 95 females = 3.65 +.
P. E. M [sub.] 141 males = ± 0.198 +.
P. E. σ [sub.] 141 males = ± 0.14008 +.

TABLE VIII. — LENGTH OF BILL.

AREA.	MATERIAL.	MODE.	MEAN.	STANDARD DEV.
Entire region .	164 males.		12.01 + mm.	0.71 + mm.
	112 females.		11.71 + mm.	0.63 + mm.
Austroriparian .	69 males.	12.1 mm.	12.58 + mm.	0.59 + mm.
	30 females.	12.1 mm.	12.17 + mm.	0.51 + mm.
Appalachian . .	38 males.	11.8 mm.	11.63 + mm.	0.56 + mm.
	29 females.	11.9 mm.	11.26 + mm.	0.54 + mm.
Campestrian . .	25 males.	12.1 mm.	11.84 + mm.	0.63 + mm.
	20 females.	12.1 mm.	11.57 + mm.	0.51 + mm.
Sonoran . . .	32 males.	12.1 mm.	11.96 + mm.	0.54 + mm.
	35 females.	12.1 mm.	11.76 + mm.	0.53 + mm.

C V. of 164 males = 5.89 +. *C V.* of 112 females = 5.35 +.
P. E. M. [sub.] 164 males = ± 0.0374 +.
P. E. σ [sub.] 164 males = ± 0.0264 +.

TABLE IX. — DEPTH OF BILL.

AREA.	MATERIAL.	MODE.	MEAN.	STANDARD DEV.
Entire region .	126 males.		9.27 + mm.	0.42 + mm.
	85 females.		8.95 + mm.	0.41 + mm.
Austroriparian .	54 males.	10.0 mm.	9.54 + mm.	0.38 + mm.
	22 females.	9.2 mm.	9.32 + mm.	0.37 + mm.
Appalachian . .	21 males.	8.8–9.0 mm.	8.95 + mm.	0.30 + mm.
	19 females.	8.8 mm.	8.90 + mm.	0.36 + mm.
Campestrian . .	19 males.	9.0 mm.	9.08 + mm.	0.28 + mm.
	14 females.	8.8 mm.	8.8 + mm.	0.30 + mm.
Sonoran . . .	32 males.	9.0 mm.	9.12 + mm.	0.44 + mm.
	30 females.	8.8–9.0 mm.	8.78 + mm.	0.36 + mm.

C.V. of 126 males = 4.57 +. *C.V.* of 85 females = 4.61 +.
P. E. M. [sub.] 126 males = ± 0.0252 +.
P. E. σ [sub.] 126 males = ± 0.0178 +.

TABLE X. — MELANISM OF TOP OF HEAD.

AREA.	MATERIAL.	MODE.	MEAN.	STANDARD DEV.
Entire region .	144 males.		83.57 + %.	3.00 + %.
	99 females.		83.66 + %.	3.19 + %.
Austroriparian .	57 males.	86%.	86.17 + %.	1.69 + %.
	24 females.	88%.	87.25 + %.	1.81 + %.
Appalachian . .	33 males.	80%.	82.24 + %.	2.68 + %.
	25 females.	80%.	82.32 + %.	3.03 + %.
Campestrian . .	21 males.	80%.	80.67 + %.	1.13 + %.
	17 females.	80%.	80.94 + %.	1.78 + %.
Sonoran . . .	33 males.	82%.	82.24 + %.	2.4 + %.
	33 females.	82%.	83.45 + %.	2.38 + %.

C.V. of 144 males = 3.58 +. *C.V.* of 99 females = 3.81 +.
P. E. M. [sub.] 144 males = ± 0.1686.
P. E. σ [sub.] 144 males = ± 0.119 +.

TABLE XI. — MELANISM OF UPPER TAIL COVERTS.

AREA.	MATERIAL.	MODE.	MEAN.	STANDARD DEV.
Entire region .	142 males. 104 females.		53.13 + %. 47.98 + %.	15.42 + %. 18.99 + %.
Austroriparian .	55 males. 25 females.	70%. 65%.	67.22 + %. 66.2 %.	5.02 + %. 8.16 + %.
Appalachian . .	34 males. 20 females.	60%. 60%.	58.38 + %. 53.2 %.	9.3 + %. 6.4 + %.
Campestrian . .	18 males. 17 females.	30%.	25.28 + %. 28.25 + %.	11.4 + %. 14.8 + %.
Sonoran . . .	35 males. 43 females.	35%. 30%.	40.43 + %. 38.26 + %.	13.01 + %. 13.37 + %.

CV. of 142 males = 29.02 +. *CV.* of 104 females = 39.58 +.
P. E. M. [sub.] 142 males = ± 0.873 +.
P. E. σ [sub.] 142 males = ± 0.617 +.

TABLE XII. — MELANISM OF BREAST.

AREA.	MATERIAL.	MODE.	MEAN.	STANDARD DEV.
Entire region .	124 males. 98 females.		31.21 + %. 36.58 + %.	11.07 + %. 10.51 + %.
Austroriparian .	42 males. 27 females.	25%. 35%.	23.81 + %. 32.41 + %.	6.66 + %. 10.36 + %.
Appalachian . .	35 males. 23 females.	35%. 40%.	43.28 + %. 47.61 + %.	9.4 %. 8.71 + %.
Campestrian . .	17 males. 18 females.	25%. 35%.	27.65 + %. 31.39 + %.	7.27 + %. 6.63 + %.
Sonoran . . .	30 males. 30 females.	25%. 35%.	29.5 %. 35.0 %.	6.65 + %. 7.30 + %.

CV. of 124 males = 35.48 +. *CV.* of 98 females = 28.72 +.
P. E. M. [sub.] 124 males = ± 0.6709 +.
P. E. σ [sub.] 124 males = ± 0.474 +.

Because of limited time I was unable to obtain measurements of culmen curvature for more than forty-seven individuals (partly males and partly females), a number so small that a rather large probable error is found for coefficients of variability.

The results obtained are given in the following table.

TABLE XIII. — CURVATURE OF CULMEN.

	23°	24°	25°	26°	27°	28°	29°	30°	31°	32°	33°	34°	35°	36°	MEAN.
Austroriparian	1					1	1	1	2						28.66° +
Appalachian .				3	2	2	4	5	2	3	1				29.32° +
Campestrian .					2	4	1	1	1	0	2	2	2	1	31.00°
Sonoran . .								1	1				1		32.00°
Total. . . .	1			3	4	7	6	8	6	3	3	2	3	1	29.936°

Austroriparian $\sigma = 2.74^\circ +$. *CV.* = 9.58 +. *P. E.* $\sigma = \pm 0.534 +$.
Appalachian $\sigma = 2.03^\circ +$. *CV.* = 6.92 +. *P. E.* $\sigma = \pm 0.206 +$.
Campestrian $\sigma = 3.12^\circ +$. *CV.* = 10.06 +. *P. E.* $\sigma = \pm 0.372 +$.
Sonoran $\sigma = 2.16^\circ +$. *CV.* = 6.75. *P. E.* $\sigma = \pm 0.594 +$.
Total $\sigma = 2.74^\circ +$. *CV.* = 9.15 −. *P. E.* $\sigma = \pm 0.191 +$.

The shrikes of the Austroriparian subprovince, mostly Florida birds, are seen to have a mean curvature 3.34° — less than that of the Sonoran birds measured. With so few individuals precise statements as to curvature are not very reliable, but I believe that the figures given are approximately correct for the four subprovinces represented. The series of measurements of the curvature of the culmen when half the base line *A B* (Fig. 3) was used as the chord gave the following constants and coefficients of variability for the forty-seven individuals measured in the first series: Mean, 26.34° +; Mode, 27°; $\sigma = 2.33^\circ +$; *CV.* = 8.85; *P. E.* $\sigma = \pm 0.162 +$.

The angles obtained from the second series of measurements were never greater than those from the first series. The greatest deviation from the first set of measurements was 7°, which occurred in four cases. All the deviations are indicated in the following table:

0°	1°	2°	3°	4°	5°	6°	7°
4	2	6	10	10	7	4	4 individuals.

It was impossible to get as accurate results in the second series, because of greater difficulty in determining the actual point of tangency, but the results obtained show that there is a fairly close correlation in the curvature for various parts of the bill; that is, where the culmen is much curved in one place, it is likely to have correspondingly strong curvature at other points.

The only selection exercised in the whole work was employed in the estimation of curvature, individuals which appeared on inspection to be typical for their respective subprovinces having been chosen, since it was impossible, for want of time, to photograph the entire series.

C. Discussion of Results and Conclusions.

Although computations have been carried out to the third or fourth decimal place, figures beyond the second decimal place are not given, as they would imply a degree of precision which is not attainable in an investigation of this kind. For example, the mean length of wing for 168 male shrikes is seen to be (see Table VI) 99.09 + mm. It often happened in making correlations that, because of general wear or some special mutilation to a single character, some individuals could not be included in a correlation table. This has happened especially often in the case of the tail, so that in the correlation table for wings and tails only 140 of the 168 male shrikes could be included. The mean for the left wings of the series of 140 shrikes was found to be 99.06 mm. (see Table I), a result which differs from the mean of the whole lot (168) by 3 in the second decimal place.

The modes and means given for the melanism of the breasts of Appalachian-subprovince shrikes show a percentage which, though higher than in other subprovinces, is not as high as would have been the case had not a large proportion of individuals come from localities intermediate between the Campestrian and Appalachian subprovinces. Shrikes from New England were found to have 50–60% of melanism for the breast. An analysis of material shows that only five skins were obtained from New England, whereas Illinois alone is represented by thirteen. This lack of equalization in the sources of material prevents certain desirable interpretations of tendencies towards minor variations.

To my mind one of the most important results reached is the determination of the relative variability of different characters in a group of birds representing geographical areas of considerable size. The coefficients of variability (Tables VI–VII) indicate for the wing and tail a variability of less than 4 in length. The bill is somewhat more variable, as is shown by a coefficient of 5.89 + for the length of bill in a series of 164 males. Color, as would be expected, is much more subject to variation. The upper tail coverts and breast are the most variable; but the coverts furnish only a very small part of a bird's coloration, and the color of the breast was the character which it was the most difficult to measure satisfactorily, especially as advanced age and the condition of the plumage are factors of possible importance which I have found it difficult to consider. Fortunately for this particular study, shrikes do not change much after the first winter plumage is obtained.

I believe that migrans is as worthy of recognition as gambelli. Whether it is profitable to encumber nomenclature with the names of these races, based on slight variations, is a question which is worthy of further consideration.

The power of discriminating fine shades of color varies in different persons, and it can be highly developed by education. At the present time there is much activity among certain systematists in the production of new subspecies for geographical varieties, which long experience and special adeptness enable them to distinguish. A variation, no matter how slight, that can be correlated with geographical range is considered to warrant an addition to nomenclature; but the discovery and description of geographical races can be carried on almost *ad infinitum*.

Birds, because of their powers of flight, might be expected to be less subject to the factor of isolation than non-migratory animals, but the tendency to return in spring to the same breeding place must, in some species at least, be conducive to the formation of numerous local variations, or family characteristics, whose recognition is a matter of power of discrimination on the part of the systematist.

It seems highly desirable that the question of limiting the establishment of new subspecies or varieties by some generally accepted criteria be considered.

I do not argue for the universal use of the method of the "Precise Criterion," but I believe that it is both desirable and practicable to employ it in certain problems of taxonomy, such, for instance, as the one just discussed. The ordinary work of classification, perhaps, does not at present require the precision in treatment furnished by purely quantitative methods, but problems of race distinction, I believe, need the precision of the "Precise Criterion." The contention that quantitative methods are less useful than those ordinarily employed because of the large amount of material required, is mischievous, for it argues that generalizations professing precision are possible by methods which are not precise. The problems of finer classification can be properly settled only by the use of a large amount of material, whatever the methods used.

V. SUMMARY.

Quantitative methods have here been applied to the study of variation in the smaller shrikes of North America, and the following variable characters have been measured:

1. Length of wing.
2. Length of tail.
3. Length of bill.
4. Depth of bill.
5. Curvature of culmen.
6. Color of dorsal surface of head.
7. Color of upper tail coverts.
8. Color of breast.

The three color areas have been found to vary principally in the amount of melanism present. A series of 294 shrike skins from various parts of the United States, Mexico, and southern Canada have been studied and measurements of these skins have been classified.

Coefficients of variability have been determined as follows for the whole series.

	MALES	FEMALES
Length of wing	2.81 +	2.69 +
Length of tail	3.43 +	3.65 +
Length of bill	5.89 +	5.35 +
Depth of bill	4.57 +	4.61 +
Melanism of top of head	3.58 +	3.81 +
Melanism of upper tail coverts	29.02 +	39.58 +
Melanism of breast	35.48 +	28.72 +
Curvature of culmen . . 47 males and females	9.15 −	

Florida shrikes were found to have relatively large bills and long tails.

A large percentage of melanism has been found for the top of head and the back of shrikes from the south central states, while shrikes from the vicinity of Colorado and Arizona have a relatively small percentage of melanism for all three color surfaces measured.

I wish to make acknowledgments to Dr. Davenport for supervision of the work and helpful criticisms, and to Dr. Mark for suggestions and revision of the manuscript.

CAMBRIDGE, MASS.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE.

E. L. Mark, *Director*.

⁂ Abbreviations used:—

B. M. C. Z. . . . . for Bull. Mus. Comp. Zoöl.
P. A. A. . . . . for Proceed. Amer. Acad. Arts and Sci.
P. B. S. N. H. . . . . for Proceed. Bost. Soc. Nat. Hist.

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59.18.8: 11.8: 6

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MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD
COLLEGE. E. L. MARK, DIRECTOR.

No. 122.

THE DEVELOPMENT AND FUNCTION OF REISSNER'S FIBRE, AND ITS CELLULAR CONNECTIONS.

A PRELIMINARY PAPER.

BY PORTER EDWARD SARGENT.

WITH TWO PLATES.

REPRINTED FROM THE PROCEEDINGS OF THE AMERICAN ACADEMY OF
ARTS AND SCIENCES, VOL. XXXVI., NO. 25.

CAMBRIDGE, MASS., U. S. A.
APRIL, 1901.

59.53.84: 14.85: 11.85

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE
MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD
COLLEGE. E. L. MARK, DIRECTOR.

No. 123.

THE OTOCYST OF DECAPOD CRUSTACEA: ITS STRUCTURE, DEVELOPMENT, AND FUNCTIONS.

BY C. W. PRENTISS.

WITH TEN PLATES.

FROM THE BULLETIN OF THE MUSEUM OF COMPARATIVE ZOÖLOGY
AT HARVARD COLLEGE, VOL. XXXVI. NO. 7.

CAMBRIDGE, MASS., U. S. A.
JULY, 1901.

FEB 26 1902

[FROM THE BULLETIN OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT
HARVARD COLLEGE, VOL. XXXVI., No. 7, JULY, 1901.]

No. 7. — *Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the direction of* E. L. MARK, No. 123.

THE OTOCYST OF DECAPOD CRUSTACEA: ITS STRUCTURE, DEVELOPMENT, AND FUNCTIONS. By C. W. PRENTISS.

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INTRODUCTION.

Since the appearance of the admirable paper by Hensen ('63) on the auditory organs of decapods, a period of thirty-seven years has elapsed, a period rich in zoölogical discoveries and improvement in general technique. The great advances made in comparative neurology by means of modern methods have reopened to investigators fields for research hitherto considered exhausted. The zoölogist of the present time is thus enabled to reap a second crop on ground already carefully gleaned, and to harvest results as important as those originally obtained.

The physiological work of Hensen's paper has been continued in recent years by various investigators. But aside from the paper by Bethe ('95) on the otocysts[1] of the schizopod Mysis, little work has been done on the morphology of the decapod ear since 1863.

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[1] Throughout this paper the terms otocyst, statocyst, ear, and auditory sac will be used synonymously to designate the auditory organ, so-called, of Crustacea.

To throw more light on our knowledge of the vertebrate ear, comparative study of the (perhaps) analogous organ found among invertebrates may be of great practical value. For by such comparative study zoölogists have been enabled to solve many perplexing questions which might otherwise have proved too difficult for solution.

The present study was undertaken with this practical bearing of the subject in mind, and with the hope that by the aid of modern neurological technique it would be possible to go deeper into many undecided questions than Hensen could.

The work is necessarily twofold in its scope, owing to the inseparable nature of the morphology and physiology of the auditory organ. We have, first, to obtain more accurate knowledge concerning the structure, innervation, and development of the decapod otocyst. In doing this especial attention must be given to the innervation, which must be compared with that of other sense organs in decapods. And, secondly, we must determine from evidence obtained by others in the past, and from additional physiological experiment, whether we are justified in ascribing a true auditory function to this much discussed apparatus.

PART I. — MORPHOLOGY.

A. HISTORICAL SURVEY.

Although the literature up to Hensen's time is well summarized by him, yet it may be worth the while to take a glance at what has been done, touching upon only the more important works, however, as a fairly complete list of authors is appended in the Bibliography.

The earliest notice of an ear in Crustacea is that of Minasi, a Dominican monk, who in 1775 attributed the sense of hearing to Pagurus, the hermit crab, and described as the auditory apparatus what is now known as the green gland or excretory organ of decapods. The organ supposed to subserve the function of hearing was thus from the very first misplaced, and its identity was in doubt even up to the time of Häckel (**'57**) and Leydig (**'57**), who were the first to rectify the erroneous ideas which existed in regard to the functions of the green gland and the otocyst.

The true sacs were, however, discovered and described as early as 1811 by Rosenthal (**'11**). He mentions the cavity, its opening, and nerve; but it was left for Treviranus (**'02–'22**, Bd. 6, pp. 308–310) to discover the sand, or otoliths, present in the otic chamber.

The first good description of the organ, accompanied by figures, was given by the Englishman Farre ('43), who carefully dissected the otocysts of the crayfish (Astacus fluviatalis), the European lobster (Astacus marinus), the hermit crab (Pagurus), and the rock lobster (Palinurus quadricornis).

The organs were found by Farre to be situated in the basal segment of the inner antennæ (antennules), the thin dorsal membrane of which in A. marinus he compared to the fenestra ovalis of the vertebrate ear. The openings of the sacs were always found to be large enough to admit the otoliths, which rest upon auditory bristles. The otoliths were, he maintained, merely grains of sand. The auditory bristles were briefly described, and their semi-circular arrangement noted; a nerve was traced from the brain to the ventral surface of the otocyst, where it formed a plexus. In Farre's opinion separate fibres probably supplied the bases of the different hairs. While the otocysts of the lobster, crayfish, and hermit crab were of relatively large size, nearly filling the basal segment of the antennule, their openings were very small and well guarded by a "chevaux de frise" of bristles. In Palinurus the organ was apparently degenerate; the sac small, shallow, with very large opening, and the auditory hairs sparse and irregularly arranged. The otoliths were of large size and few in number. The whole apparatus was held by Farre to be a delicately modified tactile organ, and he doubted if a *true auditory function* could be ascribed to it.

During the next twenty-five years otocysts were discovered and examined in various decapods by Souleyet ('43), Von Siebold ('44, '48), Leuckart ('53, '59), Frey und Leuckart ('47), Huxley ('51), Leydig ('55, '57, '60), Bate ('55, '58), Hensen ('63), Sars ('67), and Lemoine ('68). Leuckart und Frey ('47) briefly described the sacs which they found in the endopod of the last abdominal appendages of Mysis, mentioning the otolith and auditory hairs.

Leuckart ('53) made a comparative study of the otocysts in many crustacean forms. He divided them into two groups: — Those having (1) closed sacs with one otolith, and (2) open sacs with many otoliths. Leuckart's general descriptions agree with those of Farre.

Kroyer ('59) devotes a few pages of his monograph on Sergestes to a comparative account of this organ in different Crustacea. He follows Leuckart's method of grouping. To the first type (closed sacs, and one otolith) belong such forms as Lucifer, Sergestes, Mysis, and Phyllosoma. In the second group (open sacs and many otoliths) are placed Homarus, Astacus, and Palinurus. In the opinion of Kroyer, Farre erred in considering the otoliths simply particles of sand; for sometimes the sacs are closed, and again the openings are often too small to admit the passage of the otoliths from the exterior. They must be, then, deposits of calcium carbonate secreted by the animals themselves.

Hensen's ('63) account of the otocyst is far more complete than any other, and a fairly extensive review of his paper is necessary for the sake of later comparisons. He worked mostly with freshly collected animals, although some twenty-four species were studied from alcoholic material. His principal methods were dissection and maceration, some few crude sections, however, being made. The paper is divided into an anatomical and a physiological part. The latter portion will be reviewed, along with other papers of a similar nature, in Part II of this paper.

The elementary parts of the typical auditory organ are described by Hensen ('63, p. 326) thus: "Der Gehörapparat der höheren Krebse besteht nun, kurz gesagt, darin, dass, von der Endganglie eines Nerven ein feiner Faden in ein Chitinhaar hineintritt, und an einen eigenthümlich gebildeten Theil der Haarwand sich festsetzt. Diese Haarwand ist so locker mit der Schalenhaut verbunden, dass sie bei entsprechenden Tönen recht bedeutende Schwingungen vollführen kann und vollführt. Das Haar selbst geht zuweilen noch in oder zwischen Steine hinein."

Crustacea he divides into four classes according to the condition of otocyst and otoliths:—

1. Sacs closed, with one otolith: example, Mysis.
2. Sacs closed, without an otolith: all Brachiura.
3. Sacs open, many otoliths: Astacus, Palæmon.
4. No sac nor otoliths, but free auditory hairs.

Otoliths. In confirmation of Farre it was found that the otoliths of decapods having open ear sacs were mainly composed of grains of sand. This was proved by chemical tests, and by keeping newly moulted animals (Palæmon) in filtered water to which uric acid crystals had been added. Examination of the otocysts some time after moulting showed the presence of these crystals in the sac. In larger forms, such as the lobster and crayfish, the sand particles are spread over the whole basal surface of the ear sac. In shrimps and prawns they are more closely aggregated. The single otolith found in Mysis flexuosus is described at length, but as this account has been corrected by Bethe ('95), it will be referred to later in connection with Bethe's work.

The *Otocyst* (Hörblase of Hensen) is described in general as a roundish or ovoid cavity, lined with chitin; the opening, if any, is always dorsal, and varies greatly in size. It is found in the basal segment of the first antennæ of all decapods, and in the endopod of the sixth or last abdominal appendage of the schizopods. The sac is closed in the Brachiura and Schizopoda, but open in most Macrura. The otocysts of Crangon, Palæmon, Hippolyte, Mysis, and Carcinus mænas are described in more detail, but no good figures or sections are given.

Auditory Hairs or Bristles. Hensen gives the first and only good description of these. They differ from common tactile hairs in that the hair shaft is not directly connected with the wall of the sac, but a thin chitinous membrane intervenes, forming a small hollow sphere. It is this "spherical membrane" which allows the great freedom of movement necessary for the shaft in its response to sound vibrations. A peculiar process, the "lingula," projects from the inner wall of the base of the shaft into the spherical membrane, and to this the nerve fibre is attached. The hair shaft is generally plumed, as in tactile hairs, with delicate chitinous filaments.

In A. marinus the hairs are plumed and are nearly one millimetre in length. They are here very numerous, 468 having been counted in one case, and are arranged on the floor of the otocyst in four parallel semicircular rows.

A. fluviatalis has a much smaller number of hairs, but the same general arrangement; Crangon, a row of only seven or eight; these are more attenuate than in either of the above forms, but are 0.75 mm. in length.

Palæmon antennarius has about 40 hairs, arranged in a half-oval or horseshoe shape, the break in the oval being posterior. The hairs themselves are peculiar in having their shafts bent at a sharp angle. The portion of the shaft above the bend is much longer and more attenuate than the basal part, and is also heavily plumed. These plumed ends project toward the centre of the horseshoe, and intertwine. Their length is about 100 μ and their greatest diameter 3.8 μ. The hairs of Hippolyte and Mysis strongly resemble those of Palæmon, but they are embedded in the single otolith and are therefore unplumed.

Carcinus mænas has about three hundred auditory hairs. They are grouped into three classes: — 1. Hook hairs (Hakenhaare): the shaft hooked and with a plumed tip, about thirty in number, 50 μ long, similar to the otolith hairs of Macrura. 2. Thread hairs (Fadenhaare): long, filamentous, plumed at very tip, a single row of about 46, each 338 μ long, 3 μ in diameter. 3. Tuft hairs (Gruppenhaare): short, blunt, and unplumed, about 200 in number, occurring in a single large group.

Hensen also found on the appendages of some decapods free hairs which closely resembled auditory bristles, and are described as such by him. Crangon especially, which has few hairs in the otocyst, is supplied with many of these so-called "free auditory hairs." They are also numerous in Mysis and Palæmon.

Innervation of the Otocyst. In Palæmon Hensen traced the nerve of the first antenna from the brain. A large branch of this nerve runs to the ventral side of the otocyst, where the fibres separate, each enlarging into a ganglionic cell and then proceeding to the base of a hair. Each of these terminal fibres ("Chordæ" according to Hensen) then enters the pore beneath a hair, passes through the spherical membrane to the lingula, or process from the base of the hair shaft, and makes itself fast to this. In his own words (Hensen, '63, p. 368): "Dieser eigenthümliche Faden, den wir als Chorda bezeichnen, läuft eine kürzere oder längere Strecke weit bis zu einem Hörhaare hinfort, und geht durch die Mitte des Porenkanals und der Haarkugel bis zur Lingula hin, an die er sich festsetzt." Essentially the same conditions were found by Hensen in Carcinus mænas and in Mysis. He also found nerve fibres supplying the tactile bristles which are present on all parts of the decapod body.

Formation of New Hairs (Haarwechsel). New hairs are not formed inside the old, but beneath the chitinous wall; and instead of developing from a single matrix cell, as was supposed, Hensen found that each was the product of a great number of cells. A new layer of chitin is formed beneath the old, and *under* this new layer, but continuous with it, the new hairs are formed as double-walled (i. e. invaginated) tubes. The new chitin wall is compared to the hand of a glove. If the fingers of the glove be turned partially outside in, so as to leave only their tips projecting, the condition would represent that of the hair tubes just before the moulting of the old shell. The tips of the newly formed hairs become attached to the shaft of the old hair, into which they project some distance, and as the latter are detached at ecdysis, the new hairs are pulled out. Nerve fibres were found running into the very tips of the new hairs. Hensen's theory is, that at moulting, the old nerve fibre, becoming more highly refractive and resembling chitin, is, upon the detachment of the old hair, drawn out through the apex of the new one, and that before this event a new fibre is formed. This theory, however, is not easily reconcilable with his statement that the nerve fibres attach themselves to the lingula at the base of the hair shaft.

The remainder of this part of his paper is devoted to brief descriptions of the otocyst as found in some twenty-four different species of Crustacea.

To this is added a table, embracing all the forms which have been studied, giving the names of the different investigators, and the conditions, as to number and size, of both the auditory hairs proper (Otolithenhaare) and the "free auditory hairs" found on the antennæ and abdominal appendages.

Lemoine (**'68**) compares the otocyst of the lobster with that of the crayfish. His descriptions are similar to those of Farre (**'43**), but his figures are poor. The thin dorsal wall of the basal segment of the first antenna, which covers the ear sac, he calls the tympanic membrane of the lobster. The opening of the sac is overlooked, and the otocyst described as closed. Thus, as the otoliths cannot come from without, Lemoine's theory is that they are exfoliations from the calcified walls of the sac, — an absurdly impossible assumption, as the thin chitinous walls of the otocyst are not calcified. In the case of the crayfish, he notes nothing new except that there is a membrane at the base of each hair shaft, separating its cavity from that of the spherical enlargement on which the shaft stands. This membrane acts as an ear drum, taking the place of the large tympanic membrane described for the lobster.

Garbini (**'80**) discusses very briefly and incompletely the sense organs of Palæmonetes varians. The figures of the otocyst are extremely crude considering the date of the work, and simply confirm the conditions found by Hensen in Palæmon.

Vom Rath (**'87, '88, '91, '94**) does not make the sharp distinction between auditory and tactile hairs that Hensen does, holding that the two kinds grade insensibly into each other, the auditory hairs being simply slightly modified tactile organs. All sensory bristles of Crustacea can be divided into two chief groups: —

(1) Tactile or auditory hairs, with long, plumed shaft, the base of which is attached to the body wall by a delicate membrane of chitin, often spherical in form. Differentiation is thus towards freedom of movement in response to tactile or vibratile stimuli; (2) taste or olfactory hairs, having a short blunt shaft, thick-walled at the base, but with either a small pore or thin permeable membrane at its distal end, by means of which chemical substances in solution can come into direct contact with the nerve endings. The nervous apparatus of these hairs is the same in both cases for all decapods. The sweeping statement is made, that *beneath every sense hair there lies, either in the hypodermis, or removed some distance from it, a group of bipolar ganglion cells.* From each of these cells a fibre is given off peripherally, and these, *forming a strand, enter the base of the hair, ending only at its very tip.*

Claus ('75, '91) agrees with Vom Rath as to the nerve ending, but maintains that there is only *one* ganglion cell sending its process through a *group* of *matrix cells* into the hair. A misunderstanding as to the relations of the ganglion and matrix cells forms the basis of several controversial papers.

Retzius ('90, '92, '95) concludes in his last paper ('95) that there may be several ganglion cells to a single sensory hair. The number may indeed vary from one to many. He was unable by any method to trace the peripheral nerve fibres further than the base of the hairs. Nerve endings, which he described in his first paper ('90) as extending into the hair shaft, he afterwards ('92) frankly acknowledges to be artifacts.

Bethe ('95^a), in his admirable little paper on the otocysts of Mysis, clears up by modern methods many points, and corrects some of Hensen's erroneous descriptions. The sac in Mysis is ellipsoidal, and pointed posteriorly, while from its floor rises a sensory cushion bearing the hairs. This cushion is tilted outwards and ventralwards 45°, the right and left cushions thus being perpendicular to each other. The sac is open, not closed as described by Hensen; the narrow aperture is concealed by the overlapping walls of the otocyst. Borne on the sensory hairs is the large otolith, oval as seen from above, kidney-shaped in side view; its greatest diameter 0.3 mm., the other dimensions being 0.25 mm. and 0.15 mm. It is composed of a more or less organic core, about which concentric layers of calcium fluoride are deposited. The tips of the sensory hairs are embedded in this inorganic layer, and penetrate to the core of the otolith. The layers of calcium fluoride are probably deposited from the sea water. The sixty sensory hairs are arranged in a single row, so as to form two thirds of a circle, the break in the line being posterior and toward the median plane of the animal. At one end of the curve five hairs are grouped together, and at the other end there is an irregular double row. Though much like the auditory hairs of Palæmon, their tips, embedded in the otolith, are unplumed. Only *one ganglion cell* to a hair was found, sending a distal process into the base of each shaft. A double row of matrix cells lies just beneath the single row of hairs, and could easily be mistaken for ganglion cells. Vom Rath may have made this mistake, thus getting a multiganglion-celled condition for each hair.

The otocyst begins to develop before the appendage is fully formed. An invagination of the dorsal ectoderm takes place, producing a shallow depression; this enlarges while the opening gradually closes. Certain of the hypodermis cells elongate to form the matrix cells which later produce auditory hairs. The latter are formed only after hatching.

Herrick ('95) mentions the auditory organ only in connection with the development of the lobster. The otocyst becomes prominent at the third larval stage, appearing as a shallow depression bordered with short setæ and containing a few grains of sand. The depression gradually enlarges, forming in the fifth stage a sac, the aperture of which decreases in size with successive moults, until the adult condition is attained.

Bethe ('95, '97) has traced the auditory fibres of Carcinus mænas centrally to the neuropil of the first antenna, where they end in delicate fibrillations. Some of these fibres may also end in the globulus.

From this review of the literature, it is seen that little has been done on the finer anatomy of the otocyst. Hensen's work, once considered exhaustive, will not suffice at the present time. The organ of Brachyura has not been touched upon since Hensen's dissections, while our knowledge as to the innervation of the different sensory hairs of Crustacea is left in a very hazy, confused state, since the exact condition of the peripheral endings is not firmly established, Claus, Vom Rath, Retzius, and Bethe each holding different views. The question remains unsettled as to whether the manner of innervation is the same for all the sensory hairs. G. H. Parker ('90) has clearly shown that the optic nerve in Crustacea is highly differentiated; but all the other sense organs have, according to Vom Rath, the same manner of innervation, even though they differ in function as much as the so-called auditory and olfactory bristles.

All the investigators of the crustacean otocyst, Bethe alone excepted, carried on their work under the impression that they were dealing with an auditory organ. This certainly prejudiced them in drawing conclusions. But for this, Hensen would never have likened the thickened wall of the crab's otocysts to the malleus of the vertebrate ear, nor made other far-fetched comparisons. A comparative study of the innervation of the otocyst, especially if supplemented by that of the olfactory and tactile bristles and the conditions in embryonic stages, cannot fail to clear up some of these questionable points.

B. OBSERVATIONS.

In the account of the morphology of the otocyst, two types will be taken for description: —

(1) *Open otocysts containing otoliths* (macruran decapods); the example will be Palæmonetes vulgaris Stimpson. The otocysts of the crayfish Cambarus affinis (Say) Girard, and of the prawn Crangon vulgaris Say, will be described in only sufficient detail to allow of comparison with Palæmonetes, and to correct any errors or omissions in the descriptions of other investigators.

(2) *Closed otocysts without otoliths* (brachyuran decapods); the sac of the green crab, Carcinus mænas Lin., will be taken as the example of this type.

For tracing out the *development* of the macruran otocyst (1), young lobsters were used instead of Palæmonetes larvæ, as it is difficult to obtain a complete series of the latter, and their small size makes them by no means favorable material for studying the embryology of the sac. Young lobsters, however, can be had in abundance during the hatching season, and are of large size; the otocyst is of the same general type as that of Palæmonetes. The development of the closed otocyst (2) was traced out in the crab for the sake of comparison with the macruran type of sac.

The research represented in this paper was carried on at the suggestion of Dr. E. L. Mark, to whom I wish here to express my thanks for his constant kindness, suggestive direction, and able criticism. I am also indebted for valuable supervision and helpful suggestions to Dr. G. H. Parker, who directed my work for one year during the absence of Dr. Mark.

1. Material.

Large numbers of Palæmonetes were obtained from the Charles River, Cambridge, at low tide. These river animals live well in either salt or fresh water, and may be kept in aquaria without running water for an indefinite period. Being so hardy, and at the same time free swimmers, they are eminently adapted for *intra vitam stains*, and for physiological experimentation.

Carcinus mænas was abundant in the soft-shelled condition, at Hadley Harbor, Naushon Id., during the months of June and July. The head of Great Harbor, Wood's Hole, was another good collecting ground.

Many soft-shelled animals were obtained by keeping young crabs in aquaria, and feeding them freely until ecdysis took place.

Lobster larvæ were hatched at the U. S. Fish Commission Station, Wood's Hole, during June and July. They were reared, but with great difficulty, up to the eighth moult. Fed on minced crab's liver they throve well; but unfortunately they also fed indiscriminately on each other.

Crangon was found in large numbers in the muddy bottom of the Charles River; crayfish were bought in the New York City markets.

2. Methods.

In sectioning, great difficulty was experienced, both on account of the thickness of the chitin, which was often calcified, and because of the siliceous otoliths, so numerous in the sacs of Macrura, and glued by secretions to the hair tips. As the otoliths are insoluble in acids strong enough to completely destroy organic tissues, the only successful remedy was to remove them mechanically. This was best accomplished by washing them out by a stream of water blown into the sac. The apparatus for this consisted of a short piece of small rubber tubing into one end of which was inserted a glass tube drawn out to a fine point. The other end of the tubing being held in the mouth, and the capillary tube inserted into the aperture of the otocyst, a stream of water was driven into the cavity of the sac with considerable force. The larger otoliths having been washed out in this way, fairly good sections could be cut.

In the crab, the difficulty in cutting the very thick calcified chitin was obviated by using soft-shelled animals. The chitin is at this stage very thin, uncalcified, and therefore more readily sectioned. Lobster and crayfish antennules were decalcified by placing them in Gilson's fluid for twenty-four hours, or in Vom Rath's platinic-osmic fixative for a week or ten days.

Of the many fixing reagents used, (1) Vom Rath's platinic-osmo-picro-acetic mixture, (2) his corrosive-picro-acetic fluid, and (3) corrosive sublimate plus 1 % acetic acid gave the best results and in the order named. The last two were followed by staining in iron haematoxylin, which gave a clear definite stain of sections as thick as 20 μ. The platinic-chloride fixative of Vom Rath was used for from three to five days, either followed or not by treatment with pyroligneous acid. In Palæmonetes and Crangon a fine differentiation of fibre tracts was obtained by using the fixative alone for three to five days, and washing out for at least two weeks in 90 % alcohol. The myelin sheath was intensely blackened, while all other tissues remained a yellowish brown.

For tracing nerve fibres, both to peripheral and central endings, *intra vitam* staining proved of most value. Different methods were employed for obtaining peripheral and central stains. A one per cent solution of methylen blue in normal NaCl was injected into the body in either case.

For peripheral endings several injections were made into the abdominal blood space, at intervals of thirty minutes. When the animals showed signs of stupefaction, a final injection was introduced into the pericardial chamber. The amount of solution injected varied from a few drops, in Palæmonetes, to five cubic centimetres, in the lobster. In from 15 to 30 minutes after the final injection the animals were usually dead. The part to be studied was then dissected out, barely covered with normal salt solution, and examined from time to time under the microscope, until a satisfactory degree of staining had been reached.

For central terminations one injection only was made, and this directly into the chamber of the heart, only a few drops of the solution being required. When the blue color was well diffused throughout the tissues (about one hour after injection), the brain was dissected out, or exposed, and examined as before. For fixation of the stain Bethe's ammonium-molybdate method for invertebrates was used. It was found to be better to leave preparations in xylol for only the shortest possible time, as this reagent diffuses the color. Preparations fixed by this method keep very well for a year or more, but after this they ultimately deteriorate, fibres originally sharp and continuous in outline becoming mere dotted lines, while the surrounding tissues take on a deep yellow hue. When both brain and otocyst were examined together, the peripheral cells and fibres stained first, then central fibres, central terminations, and ganglion cells of the brain in the order named. Sections 60–120 μ in thickness were cut, but by far the greater number of preparations were examined *in toto.* The transparency of the tissues made this possible even with the brains, a millimetre or more in thickness, of large crayfish.

To get constantly complete impregnations of both peripheral and central endings, it is necessary to expose to the atmosphere the part to be studied. The impregnation then takes place sooner, lasts longer, and affects a larger number of elements. The fixation of the color is also much better in this case, because the fluid can penetrate much more readily, and on the rapidity of its penetration depends, in a large measure, the success of fixation. Gold-chloride and Golgi preparations were useful only for supplementing and controlling the results obtained by methylen blue. Both the rapid and slow processes for silver impregnations gave fairly good preparations, but by no means as complete or constant results as methylen blue. Ranvier's gold-chloride method, in which formic acid is used for reduction, was very uncertain in its action on nervous tissue, but was quite useful in bringing out fine cell processes in the sensory hairs.

3. Structure and Development.

I. PALÆMONETES VULGARIS STIMPSON.

1. *Structure of the Otocyst.*

a. Sac. This is situated, as in all decapods except the Mysidæ, in the basal segment of the antennule, nearly filling its cavity. Its outline as seen from above (Plate **1**, Fig. 1) is nearly ovate, being well rounded posteriorly, though suddenly becoming pointed at its anterior end. In individuals of medium size (30 mm. long) its average dimensions are 0.66 mm. in length, 0.63 mm. in width, and 0.33 mm. in depth. In longitudinal section (Plate **1**, Fig. 4) its outline is somewhat kidney-shaped, its length being about twice its depth, and its ventral wall projecting into the lumen. Transverse sections through the basal portion of the antennule (Figs. 2, 3) show that the lumen of the otocyst is from one half to two thirds as wide as the antennule at this point. The chitinous wall of the sac, which is extremely thin, is continuous with that of the antennule (Fig. 3). The hypodermal cells form a single layer, except in the sensory region of the sac, where they are elongate and several layers thick. Median to the otocyst passes the antennular nerve, the cut end of which is shown at *n. at. 1* (Plate **1**, Fig. 2), and directly below it lies the large muscle of the segment. Otoliths occupy the median and posterior portion of the lumen, and nearly conceal from view the sensory hairs (Fig. 3, *set. ot.*). In parasagittal sections (Fig. 4) is to be noticed the close proximity of the brain (*n' pil. opt.*), which is not more than 0.22 mm. posterior to the sac, and projects somewhat into the base of the antennule; the sensory cushion, or prominence (*crs. sns.*), bearing the stumps of a few severed hairs, is also to be seen.

The long axis of the otocyst is not coincident with that of the antennule (Fig. 1), as its anterior end is more lateral in position than the posterior. The external aperture has the form of a pointed ellipse and penetrates the dorsal wall of the antennule; it is nearly as long as the sac itself, but does not extend quite as far back as the sac. It was described by some of the early writers as a longitudinal slit, by others as transverse; but, as Hensen points out, it is neither: its direction is oblique, and corresponds to that of the long axis of the otocyst. The opening is completely covered over by a thin fold of chitin (Figs. 1, 3, *tct.*), which extends forward and laterad to end in a sharp projection or spine. This lid-like fold (tectum) must be lifted or cut away in order to come directly at the opening of the otocyst. Figure 3 shows the position and form of the lid in transverse section, and how closely it fits over the aperture of the otocyst, while its forward projection over the anterior lip of the slit can be seen in Figure 5 (Plate **2**) at *tct.* As the chitinous lining of the otocyst is of ectodermal origin, like all other chitinous parts, it is cast off at each ecdysis, with all it contains, and a newly secreted sac takes its place.

b. The sensory cushion of the otocyst is produced by an elevation of the median and posterior portion of the floor of the sac, which projects into the lumen and gives a somewhat constricted appearance to the cyst in sagittal sections. The surface of the cushion, which is about 0.25 mm. in diameter, is not horizontal, but slants downward from the median side of the sac to its lateral wall at an angle of nearly 45° (Plate **1**, Fig. 3). This makes the sensory cushions of the right and left sides perpendicular to each other, a condition similar to that described for Mysis by Bethe ('**95**^{a}, p. 556), and of some physiological importance. The sensory hairs are borne on the sensory cushion, and for this reason the prominence has been compared to the *cristæ acusticæ* of vertebrates. The hairs, or bristles (for both names are applied to them), vary from forty-five to fifty-eight in number, and are arranged in a curved horseshoe-like row (Plate **1**, Fig. 1), the two ends of which are directed obliquely caudad and mediad. Largest at the inner end of the curve, and arranged in a single row, they grow gradually smaller toward the other end of the series, where an irregular double line is formed. Fig. 6 (Plate **2**), a transverse section through the posterior ends of the horseshoe shows the base of a single hair on the right or median side, while at the left or lateral end two bristles are seen, the lateral row being double.

Directly beneath the hairs we find, instead of the usual layer of hypodermal cells, groups of cells with elongated nuclei; these send their processes into the bases of the bristles (Plate **2**, Figs. 6,7). They are the *matrix cells*, which nourish the hair and, as we shall see later, have to do with its formation. The central region beneath the cushion is occupied posteriorly by the ganglion cells of the otocyst nerve (Plate **2**, Fig. 6, *cl. gn.*), and anteriorly by their peripheral fibres.

c. Structure of hairs. The hairs of the otocyst are peculiarly modified. Instead of being straight, as in tactile hairs, the shaft is here bent out of its course about 120°, so that its distal portion makes a sharp angle with the proximal end (Plate **2**, Fig. 8). The shaft is very long in comparison with its diameter, being from 160 μ to 200 μ in length, while only 3 to 6 μ in diameter at the base. The part of it above the bend becomes extremely attenuate, and is heavily fringed with long delicate projections (pinnules), which give it the appearance of a plume. These fine feathery tips, which always project toward the concave side of the horseshoe formed by their bases, are crisscrossed and tangled together in such a way as to form a wickerlike mesh, on which the majority of the otoliths rest (Plate **1**, Fig. 3). The hairs are not attached firmly or immovably to the wall of the sensory cushion, but an exceedingly thin-walled chitinous bulb intervenes between the shaft and the wall of the sac. This, the *spherical membrane* of Hensen, is from 6 to 12 μ in diameter, and allows the shaft, itself comparatively rigid, to sway freely on its base, as if articulated there (Plate **2**, Fig. 8, *mb. sph.*).

d. The formation of hairs has already been described by Hensen ('**63**, p. 374) in some detail. The conditions just before ecdysis were figured, but the earlier stages were not given; so a few supplementary facts may be added here. Braun ('**75**) verified Hensen's account of *Haarwechsel* in the bristles of Astacus, and himself discovered some new details.

As before stated, each sensory hair is produced by a number of matrix cells, which send their processes into the shaft. In newly formed hairs, these protoplasmic processes extend to the very tip of the hair cavity (Plate **2**, Fig. 7). In preparation for the next moult they are withdrawn nearly to the base of the hair, leaving the greater part of the hair cavity empty (Plate **2**, Fig. 9). At the same time the matrix cells from which these processes are given off sink deeper into the tissue, below the level of the hypodermis, and with other chitinogenous cells originating in the hypodermis, arrange themselves about the nerve fibre of the old bristle for the purpose of forming the new hair (Fig. 9, *cl. ma.*). This aggregation of cells is similar to the *papilla* described by Braun, but they are by no means as regular in outline and arrangement as those figured by him. In Palæmonetes this condition of the matrix cells exists for several weeks before ecdysis takes place, the new hairs being formed during this period. In adult lobsters and crayfish the time is probably much longer, whereas in larvæ it lasts but a few days. The chitin of the new hair shaft is secreted *pari passu* with that of the test, so that the two are continuous, but the hew hair is *beneath* the shell, in the region where the matrix cells have formed the *papilla*. It is secreted as a double tube, the distal end of the inner part of which projects, as the tip of the new hair, into the base of the old one. Figure 10 (Plate 3) shows the condition of affairs just before ecdysis in the endopod of the third abdominal appendage; *cta.* being the old test, *cta'*. the new one formed beneath it. Three newly formed hairs are seen as double tubes located deep in the appendage. The walls of the two tubes are continuous with each other at their lower or proximal ends, and the tip of the inner tube projects distally into the shaft of the old hair. This inner tube, the tip of the new hair, must be secreted by the delicate processes from the matrix cells which still extend up into the old hair during the period of hair formation. The outer tube, though continuous at its lower end with the inner, is secreted by two parallel rows of matrix cells, very similar to the chitinogenous cells of the hypodermis, and probably derived from them. Hensen ('63, p. 375) has well described this condition of the new hairs as resembling that of the finger of a glove turned partially inside out, the tips alone projecting. The tip of the new hair is embedded in a viscous, homogeneous substance, which is formed between the old and the new tests, either by glandular secretion of other cells or by the chitinogenous cells themselves. This substance probably corresponds to the homogeneous non-cellular membrane found by Herrick between the shells of the lobster ('95, p. 87). When the old test is shed, it adheres to the fine plumes of the new hair tip, and aided by the internal blood pressure (very considerable at the moulting period), draws the recently formed hair out into its functional position, just as one would draw out the invaginated finger of a glove by pulling on its tip. The chitin of the shaft is very soft and pliable at this time, allowing the hairs to be turned right side out with ease; indeed, this may be done artificially. But if by some accident at the time of ecdysis any of the hairs are not at once fully drawn out, the chitin hardens and they are fixed in their abnormal position.

Aside from its general interest, this peculiar method of forming the new hair is very important, as throwing light on the peripheral endings of the nerve fibres in the sensory hairs. By it certain conditions may be explained. At each moult the nerve fibres lose their connection with the old hairs, and come into relation with new ones. How these changes are brought about can best be described in connection with the innervation of the otocyst.

e. The Otoliths are borne in a rather compact mass upon the interlaced tips of the sensory hairs (Plate 1, Fig. 3, *ot'lth*). They consist of irregular grains of sand mingled with other fine mineral particles and organic detritus. The largest measure from 8 to 12 μ in longest dimension. That the greater part of them are siliceous is shown by their insolubility in strong sulphuric acid, and by the fact that they scratch glass when crushed upon it. They are renewed after each moult, for the freshly formed sac is at first without them. New otoliths are pushed in by means of the chelæ through the aperture of the sac while its walls are yet so soft and flexible as to admit quite large grains of sand. By watching animals soon after moulting it can be observed that they stir up the sand at the bottom of the aquarium in which they are confined; as soon as some particles have come to rest upon the dorsal side of the antennule, one or both chelæ are raised, and by their tips the grains of sand are pushed back under the protecting lid of the opening into the otocyst. Otocysts from which most of the sand particles had been carefully removed by forcing a jet of water into the sac were found after a lapse of two days to contain otoliths derived from iron filings which had been strewn on the bottom of the aquarium. The otoliths are often entangled in the feathery plumes of the auditory hairs, and are in this case attached to them by an organic substance, which is probably secreted by unicellular glands situated beneath the floor of the sac. No multicellular glands, such as are found in the lobster and crayfish, could be detected beneath the otocyst of Palæmonetes. Very minute canals, which are probably the ducts of gland cells, were found running through the chitin wall and some distance into the tissues beneath; they were very clearly brought out, and their tubular condition proved beyond a doubt, in silver preparations, and in those made with lead formate; but unfortunately their connection with gland cells could not be demonstrated. The functions of the otolith and the part it plays in audition, or equilibration, will be discussed in the experimental portion of this paper.

2. *Innervation of the Otocyst.*

As already noted, the brain, or supra-œsophageal ganglion, is less than a quarter of a millimetre distant from the ear sac. The nerve supplying the hairs of the otocyst is thus comparatively short, and can be traced in a single section from the central to the sensory termination. Figures 4 and 12 (Plates 1, 3) show its general course after leaving the brain. Its sensory ganglion lies directly beneath the posterior end of the sac. The nuclei of the nerve cells of the ganglion are situated about 0.25 mm. back of the hairs which they innervate, grouped irregularly together; the peripheral fibres of the cells run somewhat parallel to one another, then spread out radially to the different hairs of the circle which they supply (Plate 3, Fig. 12, *fbr. pi'ph.*).

There are three questionable points to be settled in regard to the innervation of the otocyst, and the same is true for the sensory bristles of decapod Crustacea in general.

a. Is each hair supplied by one nerve fibre and sensory cell, or by many?

b. How do the peripheral fibres terminate? Do they attach themselves to a sense cell, or to some part of the hair, or do they end free? If this latter be the condition, does the fibre terminate at the base of the hair, or at its very tip?

c. Where do the fibres end in the central nerve organ, and how?

For the determination of these questions, it is important to compare the conditions found in all kinds of sensory bristles. Because different types of hairs have been used in various Crustacea for the study of the nerve terminations, and this difference in kind of material employed by various investigators may account for the very diverse conclusions they have drawn.

All sensory bristles of decapod Crustacea can be divided into two general types:

(1) *Tactile bristles* (Plate 2, Fig. 8) have typically a long, straight, plumed, attenuate shaft, attached at the base by a thin spherical enlargement, which allows great freedom of movement.

Auditory hairs, so called, are merely modifications of these, for all gradations between the two exist. Tactile hairs are found on nearly all the appendages, and on some parts of the body.

(2) *Olfactory bristles* (Plate 4, Fig. 13, *set. olf.*, and Fig. 14) are short, cylindrical, or slightly tapering, and firmly attached as compared with tactile hairs, there being no marked basal enlargement. At the tip, the

chitin is either pierced by a pore, or ends in a thin permeable membrane, which allows substances in solution to enter the cavity of the hair. If found on the first or second antennæ, they are termed *olfactory* hairs; when on the oral appendages, *taste* or *gustatory* bristles, though their functions are probably the same.

a. Number of Nerve Elements to a Single Bristle. Until 1891 it was supposed that only a single ganglion cell and fibre-process supplied each hair. Then Vom Rath ('**91**, p. 207) asserted, that *beneath every sensory hair of crustaceans there is a large group of ganglion cells, each sending out a peripheral process, these converging and entering the base of the hair as a single large strand.* This opinion he again expressed in 1894 for all arthropods. He did not study the innervation of the otocyst, but apparently confined his attention to the olfactory type of hair, as his figures are all of unfringed bristles.

The number of elements supplying each hair of the otocyst can be determined by, first, counting the number of fibres in the auditory nerve, and the number of nerve cells connected with these fibres, and then, secondly, comparing the statistics thus obtained with the number of hairs in the otocyst. If there is but a single cell and fibre to a hair, these numbers should coincide, at least approximately. But if there are always numerous elements, as Vom Rath maintains, then the number of fibres and nerve cells should be many times that of the hairs. The number of fibres can be readily counted in a transverse section of the otocyst nerve stained intensely with iron hæmatoxylin and only slightly decolorized. The ganglion cells can be enumerated in serial sections cut in the plane of the long axes of the cells, so that their characteristic size and bipolar condition (seen in Plate **2**, Fig. 6) will readily distinguish them from the hypodermal or matrix cells. From many such counts, the number of nerve elements was found to be approximately equal to that of the hairs. For example, in one otocyst there were 55 hairs, 53 fibres in the nerve supplying them, and 58 cells connected with these. The number of cells could not be determined with perfect accuracy, as some cells may have been halved in sectioning. Slight variations in the numbers, however, are not of great significance, as, in order to have even two nerve elements to a hair, the number of fibres or cells must be at least twice as large as that of the hairs. Moreover, the ganglion cells are always isolated, and each is surrounded by a separate sheath; their fibres are also separated from each other. Neither cells nor fibres occur in groups surrounded by a common sheath as Vom Rath ('**92**) describes them. *In the otocyst, then, there is but one nerve element to each hair.*

In the *tactile hairs* the same methods of procedure were followed; and further evidence was obtained from methylen-blue preparations. One of these is shown in Figure 11 (Plate 3). It will be observed at once from this figure that there is only one cell and one fibre to each hair. But in other preparations of the same appendage (Plate 4, Fig. 14) from two to ten cells are found grouped together irregularly, and sending all their processes to the same bristle. When this was the case, *it was always observed, that the hair so supplied was of the short, blunt, fringeless type*, and so possibly not a *tactile* but an *olfactory* hair.

So far, the evidence has been entirely against Vom Rath's statement; but if we examine the innervation of the *olfactory bristles*, entirely different conditions will be found to exist, and in complete accord with his conclusions.

On the inner flagellum of the first antenna of Palæmonetes numerous olfactory bristles are found, arranged in rows of four or five hairs each (Plate 4, Fig. 13). The nerve cells and fibres supplying these hairs stain beautifully with methylen blue. Only single elements at first appear, but if the stain is allowed to act for a longer period, nearly every cell and fibre will become impregnated. It can then be seen that a large number of elements supply each hair. The cells are packed so closely together as to make the counting of a group difficult, but many counts upon sections stained in hæmatoxylin make it certain that more than a hundred cells may compose a single group, and supply a single olfactory hair. The cells send off each a peripheral fibre. These fibres enter the base of an olfactory hair as a single large strand, 12 to 15 μ in diameter. In Figure 13 only a few of the elements are shown; the sheath, which surrounds both cells and fibres, marks the outline of the spindle-shaped group of cells, and shows the size of the fibre strand.

The *gustatory hairs* on the oral appendages are also each supplied with numerous nerve elements (Plate 4, Fig. 14). The number is not nearly so great as in the olfactory hairs, — averaging about 10 to a hair, — nor are they so regularly and compactly grouped. They differ markedly, however, from the conditions found in tactile and otocyst hairs.

The distinctly different conditions — as regards the number of nerve elements of the hairs — found in the olfactory and otocyst bristles, seem to explain the diverse conclusions of Bethe and Retzius on the one hand, and Vom Rath on the other. The two former observers worked on the tactile type of sensory bristles, while Vom Rath, as his figures show, evidently confined his attention to the other type. The conditions which

Vom Rath found in the olfactory type he too hastily attributed to all the sensory hairs of Crustacea.

b. Peripheral Terminations. Here again we find a difference of opinion. Hensen ('**63**, p. 368) asserted that the peripheral fibre was attached to a process (*lingula*) from the base of the hair shaft. Claus ('**91**), Vom Rath ('**92**, '**94**), and Bethe ('**95**) found fibres reaching to the very tip of the sensory bristles; while Retzius ('**95**, p. 17) found no evidence of nerve terminations beyond the enlargement at the base of the hair in *decapods*, though he observed in *Entomostraca* the same conditions as did the other three investigators.

I have obtained hundreds of preparations of nerve endings in the various sensory hairs of Palæmonetes with several of the best modern nerve methods, and all furnished the same evidence. The conditions found for otocyst hairs were in *every* case as illustrated in Figures 4 and 8 (Plates **1**, **2**). The ganglion cells, as already noted, lie at some distance (0.25 to 0.40 mm.) from the bases of the hairs which they supply. The reason for this becomes obvious, when the formation of the new hairs is considered. The developing hair tube extends below the base of the old hair a distance equal to at least one-third the length of the hair, and the ganglion cells necessarily lie below the lower or proximal end of the hair tube (Plate **3**, Fig. 10, *tb. set.*). Hence they must be at least a third the length of the hair distant from its base, though they occupy a closer position directly after ecdysis than for some time before. The terminal fibres (Plate **2**, Fig. 8, *fbr. n.*), which are as long as the distance of their cells from the hairs, enlarge slightly as they near their termination, and always end in the expanded base of the hair directly below the shaft proper. There are no signs of attachment to any part of the wall of the hair, nor of fine branching of the distal end of the fibre, such as Retzius ('**90**) describes. Figure 4 (Plate **1**) shows diagrammatically one nerve element of the otocyst, the position of the ganglion cell, and the ending of its peripheral fibre in the base of the hair. In Figure 8 (Plate **2**) only the termination of the fibre, highly magnified, is given.

The elements of the *tactile hairs* end in precisely the same manner as those of the otocyst. A number of these endings are shown in Figure 11 (Plate **3**). In no case was a nerve ending demonstrated in the shaft of the hair. Thus, all the evidence of preparations goes to prove that in *both otocyst hairs and tactile hairs the nerve fibre, without branching, ends in the enlargement at the base of the hair, and never enters the shaft itself.*

In the *olfactory bristles* the cells are situated about 0.45 mm. posterior to the bases of the hairs, and their peripheral nerve fibres, stained by methylen blue, were traced *in almost every preparation, some distance into the shafts,* though in the *tactile* hairs of the same appendage no fibres could be followed further than the base. Figure 13 (Plate 4) shows the olfactory endings, some of them extending half the length of the hair shaft, but *none as far as the tip;* nor was such a condition ever found, although a great number of preparations were examined. The direct evidence of preparations shows, then, that *the peripheral nerve endings are different for the different types of hairs. The fibres terminate in the enlarged base of tactile bristles, while in olfactory hairs they end free in the shaft itself.*

This direct evidence is strengthened by other structural conditions.

(1) Owing to the rigidity of the hair shaft and its delicate basal attachment, a mechanical stimulus applied to a tactile hair would be apt to produce its strongest effect at the base. Therefore we should expect to find the nerve termination at this, the point of greatest stimulation. The innervation of the tactile hairs of vertebrates extends only to the base, yet the slightest touch of the hair tip stimulates the nerve ending.

Similarly, in the otocyst hairs the point of greatest stimulation must be at the base. The hair tips are so entangled with each other, and with the otoliths resting upon them, that a stimulus applied to one must affect them all. If this stimulus is caused by the shifting of the weight of the otoliths resulting from a change in the direction of the pull of gravity, it will affect the delicate, labile articular membrane at the base of the hairs far more vigorously than the part of the shaft attached to an otolith, or entangled with the tip of another hair which is so attached.

In the olfactory hair, on the other hand, the chemical stimulus finds access through the permeable tip, and, traversing the cavity of the shaft, comes at once into contact with the terminations of the nerve, which here, as we have seen, runs some distance toward the tip of the hair. This, then, is a condition of affairs which, in view of the function of the olfactory hairs, we should reasonably expect.

(2) The *conditions during hair formation* are very unfavorable to the assumption that the nerve fibres extend to the tips of the tactile and auditory hairs. In adult Palæmonetes, a month at least before ecdysis takes place, the matrix cells withdraw their processes to the basal portion of the hair, leaving the upper part of the shaft empty. As the

shrimp moults once in two or three months, this means that for nearly half the time the nerve fibre cannot extend further than the base of the hair. Yet the animals are apparently as sensitive to stimuli during this period as at any other. After the new hair is fully formed, and its tip projects into the base of the old hair, which has now lost all direct nerve connection, the animals still respond quickly to tactile stimulus; the impulse resulting from the stimulus is transmitted from the tip of the old hair to its base, thence to the shaft of the new hair, by which in turn it is transferred to the nerve fibre.

(3) If certain of the nerve fibres supplying the tactile hairs are stained with methylen blue just before ecdysis when the new hairs are fully formed but still deeply invaginated (Plate 3, Fig. 10, *tb. set.*), they may be traced some distance into the shaft of the *new* hair. Now, by removing with a fine needle the old test, *cta.*, the new hairs can be pulled out into their functional position. The nerve fibres, however, are not pulled out with the hair the whole distance, but remain nearly in their original relative positions, barely projecting into the bases of the hairs, a condition already pointed out in Figure 11 (Plate 3).

It is unfortunate that the investigators of these nerve endings have never taken into account the tissue changes — certainly of great importance — which occur in all Crustacea between moults.

At certain stages in their formation the delicate protoplasmic processes in the tips of the new hairs stain very sharply, and have a varicose appearance, similar to that of nerve fibres; as these project some distance into the old hairs, they might easily be mistaken for terminal nerve endings.

c. Central Terminations. By means of methylen-blue preparations the nerve fibres supplying the otocyst were traced continuously in their course from the sac to their central endings. Whole preparations of the antennules and brain could be used for this purpose, as the tissues were extremely transparent. On account of the proximity of brain and otocyst, the nerve supplying the latter is very short. It enters the anterior end of the brain lateral to the antennular nerve, the two joining as they pass within (Plate 3, Fig. 12). While the antennular nerve pursues a straight course, the other (Figs. 2, 4) descends from the sensory hairs in the floor of the otocyst, forms the sensory ganglion, and in continuing its course approaches somewhat the median plane and describes the form of an elongated letter *S*, the plane of which is dorso-ventral. Just before the two nerves unite to enter the brain, a third smaller nerve is received by the otocyst nerve on its dorsal side (Plate 1, Fig. 2, *rm. l.*). This nerve is formed by an aggregation of fibres from the tactile bristles of this segment of the antennule, and runs almost straight toward the median plane till it joins the nerve of the otocyst. The fibres of the latter enter the anterior end of the brain ventral to the optic neuropil, and median to the globulus (Plates 1, 3, Figs. 4, 12); they extend backward to near the posterior end of the central organ in an almost horizontal plane, lateral to the fibres of the antennular nerve. They end in a region just anterior and median to the neuropils of the second antennæ, branching into delicate dendritic fibrillæ, which form a well-marked neuropilar mass (Fig. 12, *fbr′.*).

Fibres supplying the tactile hairs of the basal segment of the antennule end in the same neuropil, while the main nerve to the antennule ends in a closely connected fibrillar mass just median to it. No nerve cells were found in the brain connected with the sensory fibres from the otocyst. Association elements, with large dendritic branches, put these neuropils into communication with the optic centres. One of these connecting fibres is shown in Figure 12 (*fbr. ass.*). Its cell, which supposably exists, was not stained. According to Bethe's ('97, Taf. xxviii. *an.1*) experimental work on the brain of Carcinus mænas some of the otocyst fibres should end in the globuli. He could not demonstrate such fibres, however, in his preparations of the crab's brain, nor was I able to obtain conclusive evidence of such endings in the globuli of Palæmonetes.

d. Histology of the Nerve Elements. The nerve fibres of Palæmonetes are relatively large; those of the otocyst reach their greatest size immediately before they enter the neuropil substance of the brain. At that point in their course they are from 3 to 5 μ in diameter, not including the nerve sheath. In a transverse section of the nerve the separate fibres show distinctly, as they are held apart by connective tissue.

The *fibrillar* structure was made out definitely only in methylen-blue preparations which had been well differentiated in process of fixation. The gold-chloride method of Apáthy, though tried several times, did not give a successful reaction. Fibrillæ were made out distinctly in only one preparation, though some evidences of such structure appeared in many. Figure 15 (Plate 4) shows a portion of a peripheral fibre in which many fibrils are seen running longitudinally. No single fibril was traced any considerable distance, nor could any evidence of the fibrils be found in the ganglion cells. The fibrillæ are embedded in a semi-fluid, homogeneous substance, which is the first to take up the methylen-blue stain. It has been called by Bethe ('98) the "peri-fibrillar substance." The accumulation of this fluid into drops gives the characteristic beaded appearance of methylen-blue preparations.

A distinct nucleated *myelin sheath* surrounds both the fibre and the peripheral ganglion cells of Palæmonetes. This sheath, which stains intensely black in Vom Rath's platino-osmic fixative, can be traced some distance beyond the peripheral ganglion cells toward the sensory hairs, and also centrally into the brain, where it ceases only when the fibres enter the neuropil substance. Figure 16 (Plate 4) shows a ganglion cell and its peripheral process surrounded by the sheath. Elongated, flattened nuclei occur at intervals along the walls of the sheath, curved around it and the enclosed fibre; certain of these sheath nuclei can be seen in Figure 4 (*nl. tu.*) between the ganglionic cells and the brain, though the myelin sheaths are not stained in this hæmatoxylin preparation. Quite frequently one of them may occur in close proximity to a ganglion cell. Thus are produced (Plate 4, Fig. 17) appearances which might be mistaken for a ganglion cell with two nuclei. Careful study, however, shows that one nucleus (*nl.*) lies within the cell, the other (*nl. tu.*) without, but abutting on the ganglion cell so closely as to sometimes change its form. In every instance of this kind one of the nuclei, owing to its irregular outline, its smaller size, and the curved form which it takes in adaptation to the surface of the cell, could be identified as belonging to the sheath rather than to the nerve cell.

The *peripheral ganglion cells* are much elongated and are of the typical bipolar form (Plate 4, Fig. 18). They measure from 10 to 14 μ in diameter; their nuclei are relatively large, measuring from 7 to 9 μ in diameter, and are usually ovate in outline, their length in some cases being twice as great as their diameter. One large spherical nucleolus is usually present in the chromatic network, though sometimes two or more are found. No definite structure can be recognized in the cytoplasm of the cell, nor any traces of fibrillæ; this, however, is not strange, as the cell usually stains so intensely that it would not be reasonable to expect to make out its finer structure. In methylen-blue preparations a narrow zone about the nucleus stains only faintly, the coloration becoming more intense as the periphery of the cell is approached; so here, as Bethe also found in the nerve cells of Carcinas, the chromatin granules are more numerous at the periphery of the cell cytoplasm, and nearly wanting around the nucleus.

3. *Development of the Otocyst (in Homarus americanus Milne-Edwards).*

In order that the development of the otocyst in the lobster may be more readily understood, it may be best to compare briefly its adult condition with that of Palæmonetes.

It was dissected and described by Farre ('**43**), and again by Hensen ('**63**). The sac is drawn out posteriorly into a dorso-ventrally flattened projection, the "cochlea" of Hensen. The external aperture is extremely small, guarded by bristles, and located at the median, dorsal, and anterior end of the sac, the dorsal wall of which, like the dorsal wall of the antennule, is very thin, forming the so-called tympanic membrane. On the floor, which is nearly horizontal, there is a semi-circular ridge (Plate **5**, Figs. 24, 26), which forms the sensory cushion. From this arise the otolith hairs, which have straight shafts, and number from 500 to 600. The four rows of these are so arranged as to form a semicircle, the open side of which (at the right in Plate **5**, Fig. 26), is anterior instead of posterior as in Palæmonetes. At the anterior end of the curve there is an irregular group of smaller hairs, with bent shafts. On the median wall of the sac, near its posterior end, there is an irregular double row of long thread-like hairs, with shafts heavily fringed (Fig. 26, *set. m.*). The otoliths are numerous, and rest on the area surrounded by the rows of sensory hairs, and also on the hairs themselves; the thread-like hairs are free, and float out into the lumen of the sac.

Not much has been written on the development of the otocyst in decapods. Reichenbach ('**86**), in his work on the embryology of the crayfish, figures the invagination of the "auditory sac" at an early stage in the egg. The crayfish, however, as it develops into the adult form without passing through the larval stages characteristic of most other decapods, is not a typical example. Herrick ('**95**, p. 194) alludes to the appearance of the otocyst cavity in the third larval stage of Homarus, and he shows its position at this stage in connection with the development of the first antenna. In the fourth stage it is a shallow depression containing a few otoliths and in the fifth larva its aperture begins to close.

I shall describe its condition in the first four larval stages.

a. First Larval Stage.

(Schizopod larva, without abdominal appendages.)

Sections of lobster eggs in different stages up to time of hatching showed no evidence of the otocyst in the antennule, and it became apparent that its development took place wholly in the free-swimming stages. A transverse section through the antennule of a newly hatched larva (Plate 4, Fig. 19) shows no sign of invagination in the region where the sac is to appear. But certain elongated nuclei, evidently those of modified hypodermal cells, are found grouped, two or three layers deep, beneath the dorso-lateral wall of the appendage (Fig. 19, *cl. ma.*). These elongated nuclei, viewed from the dorsal surface of the appendage, are seen to be roughly arranged in a semi-circle, like the rows of otocyst hairs in Figure 26 (Plate 5), and when traced through later stages, the position they occupy is found to be directly beneath the ridge where the sensory hairs later appear (Plate 5, Fig. 24, *set. ot.*). They are evidently, therefore, the nuclei of the matrix cells which build up by secretion the chitinous walls of the sensory hairs. These cells, like those which take part in hair formation after ecdysis, originate from the chitinogenous hypodermal cells by simply becoming elongated and sinking beneath them. A similar arrangement of matrix cells was found in the developing otocyst of Mysis by Bethe ('95^a). Numerous spherical nuclei, which stain in a manner characteristic of nerve cells, are present just below the matrix cells (Fig. 19, *n'bl.*). If traced back to the ganglionic masses of the brain, they are found to be continuous with the nerve cells of the latter, and probably originate from them.

b. Second Larval Stage.

(Second to fifth pair of abdominal appendages present.)

In this larva the first evidence of invagination is seen on the dorsal side at the base of the antennule (Plate 4, Fig. 20). The nuclei of the matrix cells are now larger, and very conspicuous at the lateral side of the transverse section, the region where the rows of hairs will later appear. Figure 22 (Plate 4) shows the anterior and posterior limits of the invagination and the fundament of the *sensory ridge*, marked by a fold in the hypodermis and chitin at *cl. ma.* The matrix cells just posterior to this fold, whose processes are directed toward it, are those which are to form the transverse portion of the hair rows. As in the first stage, nuclei of nerve cells lie immediately beneath the matrix cells, but the cytoplasm about them shows as yet no definite boundaries or outlines, nor are there any signs of nerve fibres connected with them.

c. Third Larval Stage.

(Chelæ relatively larger, uropods present.)

In this stage (Plates 4, 5, Figs. 21, 23) invagination has proceeded still further. There is a deep lateral, as well as a posterior, fold in the chitin; but the sac, if it can now be called such, is very shallow, wide-mouthed, and without sensory hairs or otoliths. From the group of matrix cells, however, the tips of embryonic sensory hairs may be made out, projecting dorsally, but covered by the chitinous floor of the sac (Plate 5, Fig. 27). Only after the wall of the sac has been shed at the next moult will they become functional organs.

d. Fourth Larval Stage.

(Form like that of adult; thoracic exopods rudimentary.)

The sac has now greatly increased in size, and nearly fills the cavity of the appendage (Figs. 24, 25). Its opening has become smaller, and is protected by numerous fringed bristles, which project from its sides (Fig. 25, *tct.*). About 200 sensory hairs are present borne on a prominent sensory ridge (Fig. 24, *set. ot.*) and arranged in three regular rows, one row less than in the adult stage (Fig. 26). The whole band bears some resemblance to a sickle. Beginning at the median side of the sac floor, the rows curving only slightly run laterally, then with a stronger bend turn forward. At the anterior end of the sac regular arrangement ceases, the hairs being grouped promiscuously. Besides these large hairs on the sensory ridge, which measure 120 μ to 150 μ in length and from 4 μ to 6 μ in diameter, there is, as in adults, an irregular row of more attenuate hairs arranged longitudinally along the posterior part of the median wall (*set. m.*, Fig. 26). They number about thirty, are on the average 140 μ in length, and have a diameter of only 2 μ to 3 μ at the base of the shaft.

Many otoliths, consisting of fine particles of sand, rest on the hairs of the sensory ridge, as in the adult condition, but do not come into contact with the attenuate bristles of the median side-wall, which project free into the liquid contents of the otocyst. The sensory ridge is much more prominent at this stage than in the adult. This, and the size of the aperture, are the chief differences between the two, and are well shown in Figure 25. The opening gradually becomes smaller in the fifth, sixth, and seventh stages, until in the full-grown animal it is almost obliterated. A fourth row of hairs, not yet developed, is formed posterior to the others at some stage later than the seventh moult, this being the oldest stage that I have studied. Except for the gradual closure of the aperture, the larvæ of the fifth, sixth, and seventh stages show the same conditions in the otocyst as the stage under consideration.

In Figure 24 (Plate 5) ganglion cells (*cl. gn.*) are seen beneath the sensory ridge. The origin of these could not with certainty be traced out in the material at command, though from the conditions found in the first stage, it is probable that they are derived from the neuroblast cells of the brain. The only evidence in favor of this view is the proximity of the brain, and the fact that at an early stage nerve cells which were continuous with the ganglionic masses of the brain were present beneath the matrix cells of the otocyst. Figure 26 shows, somewhat diagrammatically, the general innervation of the otocyst hairs of the fourth larval stage, as brought out by methylen blue. The condition is essentially that of the adult. There is but one nerve element to each hair, and the endings are in the enlarged bases. No myelin sheath is developed in either the larva or adult lobster. Central terminations of the otocyst fibres were not traced out, nor was their finer histology investigated.

The most striking point to be noted in the development of the otocyst of the lobster is the abrupt change which takes place after the third moult. The shallow, functionless depression of the third stage is converted at once into the active, well-differentiated organ of the fourth larva. This sudden leap in the development of the otocyst is correlated with an abrupt metamorphosis of the larva's general form and method of locomotion. As this correlation may have an important physiological significance, it will be discussed in detail in the theoretical portion of this paper.

II. CRANGON VULGARIS SAY.

1. *Structure of the Otocyst.*

a. Sac. The otocyst has been described only briefly by Hensen ('63). He figures the sac dissected out, and gives two sketches of the sensory hairs, and the prominence upon which they are borne.

The sac, as seen in a section passing through its middle and transverse to the long axis of the antennule, has the form of a half-circle. In a cross-section more posterior its outline is made irregular by the projection of the *sensory ridge* or cushion from its *lateral wall* (Plate 6, Fig. 28). This is an entirely different condition from that found in Palæmonetes, where the sensory cushion is basal. More irregular still is its form in frontal section, as shown at *crs. sns.* in Figure 29 (Plate 6). The dimensions of the sac in individuals of medium size (25 mm. long) are:

length 0.44 to 0.55 mm.
width 0.28 " 0.38 " (anterior to sensory ridge)
depth 0.20 " 0.22 "

It is thus relatively wider, and more shallow than that of Palæmonetes. The wall is of thin chitin continuous at the large oval aperture (Plate 7, Fig. 30) with that of the dorsal side of the antennule. The aperture is as wide and nearly as long as the sac itself; instead of a fold of chitin it has for protection a row of large fringed bristles. These are ranged close together along the posterior edge of the opening and extend their long parallel shafts beyond its anterior margin. A fine-meshed grating is thus formed, through which even microscopic organisms could not pass without displacement of the bristles.

b. The sensory cushion (Plate 6, Fig. 29, *crs. sns.*), as already noted, projects from the posterior portion of the lateral wall of the sac. Its direction is not transverse to the long axis of the sac, but it points obliquely forward and mediad. It is a *ridge* rather than a cushion, for the hairs are arranged in a short, nearly straight single row, instead of in several rows having the form of a sickle. This row of hairs, which defines the limits of the sensory region, starting at the dorsal end of the ridge, takes a course along its convex surface downward and backward, and ends where the ridge disappears, just before the floor of the sac is reached. A portion of a row of hairs is shown in the right otocyst, Figure 29, *set. ot.* (Plate 6), where the hairs anterior in position are really above or dorsal to those posterior to them. The ridge-like projection of the sensory prominence is best seen in a parasagittal section (Plate 7, Fig. 30, *set. ot.*), a hair being there shown at the apex of the ridge.

The matrix cells are essentially the same as in the hairs of Palæmonetes. They occupy the region just beneath the bristles, into which their processes extend. The space in the sensory prominence below and lateral to the matrix cells is occupied by the sensory ganglion cells, the fibres from which penetrate between the formative cells and reach the bases of the hairs (Fig. 29, *cl. gn*).

c. Structure of hairs. Arranged on the sensory ridge in the manner above described, the hairs of the otocyst are 26 in number, as shown by the average of a large number of individuals. They are largest at the upper anterior end of the row, where they measure 180 μ in length and about 9 μ in diameter at the base of the shaft. Proceeding down the line they are successively smaller, the last of the series being only 100 μ in length and 6 μ in diameter. There is a conspicuous spherical enlargement at the base of the hair shaft (Plate 7, Fig. 31, *mb. sph.*), as in the otocyst hairs of Palæmonetes. The shaft itself for about a third of its length projects straight out horizontally into the lumen of the sac. Then it bends down ventrally nearly at right angles, though the amount of curvature is different for different hairs. The larger, being in a more elevated position, usually bend at a sharper angle than those near the floor of the sac. All are heavily plumed; the pinnules are long and coarse (Plate 7, Fig. 31, *pinn.*) and often have otoliths firmly attached to them by a substance probably of glandular origin. Hensen ('63) describes the otolith hairs of Crangon, as follows: "Es steht nämlich auf die schon erwähnten Vorbuchtung eine einzige Reihe von *7 oder 8* Haaren; diese Haare reichen bis zur Kugel in die Steine hinein, *ihre Zahl erscheint viel zu gering* für deren Masse. . . . Sie sind 0.075 mm. lang, 0.0075 mm. breit und gerade aufgerichtet."

This description of these hairs is completely at variance with the conditions I have found in the American Crangon. In order to determine, therefore, whether this was a true specific difference, or due to an error on Hensen's part, a number of the European specimens, procured by Dr. Mark from Professor Herdman in Liverpool, were examined. After dissecting out the otocysts of 12 specimens, I was entirely satisfied that Hensen's description was incorrect. The hairs are precisely the same in size, form, and number as in the American variety. They have their shafts distinctly bent near the tip at angles varying from 25° to 90°; of the individuals examined none possessed less than twenty-four hairs in the sac, the average being twenty-six.

That Hensen should have made such a mistake is not strange. He himself says: "their number appears much too small for the mass [of the otoliths]." The tips of the hairs are concealed by the otoliths, and only the first third of the row would be visible from above.

d. The formation of hairs after ecdysis is identical with that of Palæmonetes.

e. The otoliths are numerous, larger than in Palæmonetes, and found mostly in the posterior part of the sac, in contact with, or even attached to, the fringed tips of the hairs. Mainly siliceous, they are taken in after each moult, being readily pushed into the large opening of the otocyst. They can be almost completely washed out by a fine jet of water introduced artificially, and if the animal so treated is then placed in an aquarium containing iron filings, or other substitute, this material will soon be used to replace the otoliths of sand.

2. *Innervation of the Otocyst.*

As in Palæmonetes, the brain is very close to the otocyst, and the nerve supplying the sac is therefore short. Its general course is shown at *n. ot.* in Figure 29 (Plate 6).

Leaving the anterior end of the brain with a bend away from the median plane, it gives off in front of the globulus a small lateral branch (*rm. l.*), which supplies the tactile bristles of the antennule. The main nerve, after passing between the globulus and the posterior end of the sac, runs forward only a short distance to the sensory prominence on the lateral side of which its ganglion lies. The peripheral fibres can be traced forward and slightly mediad from the ganglion to the bases of the otocyst hairs. The whole course of the nerve is approximately in a frontal plane, though its peripheral ending is slightly more ventral than its point of departure from the central organ. In Figure 28 (Plate 6) the transverse section of the antennular nerve (*n. at.1*) is seen to be median to the sac, while the ganglion cells of the otocyst nerve (*cl. gn.*) are lateral to it.

a. Number of Nerve Elements to a Single Bristle. There is in Crangon but one ganglion cell and fibre to each otocyst hair. The cells and fibres were counted as in Palæmonetes, and the numbers thus obtained were found to agree approximately with the number of the hairs.

Methylen-blue preparations of the olfactory nerve elements were obtained, and the conditions there brought out agreed essentially with those found in the same type of hair in Palæmonetes, large groups of nerve cells being present beneath each olfactory bristle.

b. Peripheral Terminations. Nerve fibres to otocyst hairs were never traced beyond the enlarged base of the bristle, where they end free without branching. A typical nerve element of the otocyst is given diagrammatically in Figure 29; it shows the peripheral ending of the fibre at the base of *set. ot.*

In the olfactory hairs, on the other hand, the nerve fibres in most cases could be traced up into the shaft of the hair, though never through its whole length. Thus in Crangon, as in Palæmonetes, there is a distinct difference in the innervation of the two types of bristles, both as to the number of elements, and in the manner in which the fibres end.

c. Central Terminations. Centrally the otocyst nerve ends in a position (Fig. 29) corresponding to that of the central terminations in Palæmonetes, but the fine fibrillar branching, which was brought out distinctly by methylen blue in that form, could not be impregnated in Crangon.

d. Histology of the Nerve Elements. So far as worked out, this was similar to that already described in Palæmonetes. A myelin sheath is present in Crangon as well as Palæmonetes, though it was not observed in any other decapods.

3. *Development of the Otocyst.*

This was not studied in Crangon.

III. Cambarus affinis (Say) Girard.

The otocyst of the crayfish has been figured by only Farre ('43) and Huxley ('80). The description of the former investigator was excellent for the time at which it was made. Huxley alludes to the otocyst in his work on the crayfish, and gives one figure showing the sensory region dissected out. Hensen ('63) describes the hairs of the otocyst in Astacus fluviatalis, but does not touch upon its other structures.

1. *Structure of the Otocyst.*

a. Sac. The otocyst of Cambarus (Plate 8, Figs. 37, 38), except for its smaller size, resembles that of the lobster very closely. The aperture, exceedingly small in the lobster, is here quite large, though, on account of the dense *chevaux de frise* of fringed bristles, it seems smaller than it really is. These bristles, projecting from around its margin, effectually cover and conceal the opening. It occupies the middle of the dorsal side of the antennule; its anterior margin corresponds to the anterior wall of the otocyst, and it extends back from this point nearly one-half the length of the sac. Its width is about one-third that of the otocyst (Fig. 37).

The cyst does not by any means fill the cavity of the antennule. It is rounded off in front, but sharply pointed at its posterior end, where it is very shallow (Fig. 38). Its walls are of uncalcified chitin and continuous with the very thick calcified shell of the antennule (Figs. 37, 38). Its dimensions in average-sized animals are:

Length	from	1.75 mm.	to	2.25 mm.
Width	"	1.52 "	"	2.10 "
Depth	"	0.85 "	"	1.05 "

b. Sensory Cushion. The sensory ridge, or cushion, in the base of the otocyst is not prominent, as that part of the sac floor upon which the sensory hairs are borne is but slightly elevated above the rest (Fig. 38, *set. ot.*), and, contrary to the conditions found in the two forms already described, the sensory surface is nearly horizontal, instead of being vertical or oblique. The arrangement of the hairs is shown in Figure 40 (Plate 8). Three sets can be distinguished, corresponding to the divisions of the otic nerve, — a median, a lateral, and a transverse or posterior. The first and third are nearly straight, the second sickle-shaped. The "median" set consists of a single nearly straight row, running from the posterior angle of the sac obliquely forward and mediad, back of which there are two or three shorter, irregular rows of scattered hairs. The lateral set consists of two concentric rows, which have the form of a crescent or the blade of a sickle, the handle of which is represented roughly by the nerve trunk connecting the bristles with the brain. The hairs of the outer row are much larger than those of the inner series. At the tip of the sickle blade the area covered by the bristles expands, and the hairs are arranged in 4 or 5 irregular rows. Behind the proximal end of this sickle-shaped double row of bristles is a short row of very large hairs, the posterior set (Fig. 40, *set. p.*), usually nine in number, which extends transversely across the posterior portion of the sac immediately in front of its pointed base. Matrix cells are found in the region directly beneath the hairs, as in the other forms described (Plate **8**, Fig. 37), and the nerve cells with their peripheral fibres lie below the chitin, either just within (lateral set), or slightly posterior to (median and transverse sets) the rows of hairs (Plate **8**, Fig. 40). By looking down upon the floor of the sac one can make out numerous small pores (represented in Figure 40 by minute circles), which penetrate the chitinous wall in that portion of the floor which is inclosed by the sensory bristles, especially in its lateral part. In transverse sections some of these pores are cut through, and it then appears that they connect with the ducts of multicellular glands which are located in the tissues beneath. One of these glands with its duct and pore is shown in Figure 39. It is apparently similar to the tegumental glands found in different parts of the lobster and figured by Herrick ('**95**, Cut 5, p. 77). In Cambarus these glands evidently supply the secretion which attaches the otoliths to the pinnules of the otocyst hairs.

c. Structure of Hairs. This has been described in some detail by Hensen ('**63**), to whose descriptions I have not much to add. The hairs are very similar in structure to those of the lobster. Their number varies greatly in different individuals, but is usually over 200. The straight, or only slightly curved, shaft is heavily fringed, and borne on the customary spherical base. Their dimensions are:

Length, from 65 μ to 175 μ.
Diameter, " 15 μ " 18 μ.

A transverse section of the shaft near its base has the peculiar shape shown in Figure 35 (Plate **7**). This modification of the form of its wall, found also in the otocyst hairs of the lobster, doubtless renders the shaft more rigid than if it were a simple hollow cylinder.

The shaft, as already noted, is nearly straight, but it is attached to the floor of the sac in such a way as to make a very small angle with its surface, being, in fact, nearly parallel to it. Thus in Cambarus the bending has taken place at the base, not, as in Palæmonetes and Crangon, in the shaft itself. In these two forms the tendency of the shaft to bend must be aided, if not caused, by the weight of the otoliths attached to the slender tips of the hairs. In the lobster and crayfish the modified form of the shaft makes it too rigid to thus give way, and the bending, if any, must take place at the thin, membranous basal sphere.

d. Formation of Hairs. (Not studied in Cambarus.)

e. Otoliths. These are composed of large grains of sand distributed mostly within the circle of hairs, and supported in part by them. As the sac has a large opening, they are readily taken in through it after each ecdysis.

2. *Innervation of the Otocyst.*

As the crayfish was well adapted for work with methylen blue, a large number of preparations of the sensory nerve elements were made, not only of the hairs of the otocyst, but also of the other sensory bristles. The nerve supplying the otocyst issues from the ventral surface, instead of the anterior end, of the brain, and at once passes forward with a slight lateral curvature to the pointed posterior end of the sac, beneath which its fibres spread out to the different hairs. It divides roughly into two strands, one of which passes obliquely forward and mediad to supply the median set of bristles (Plate 8, Fig. 40), while the other follows the course of the lateral sickle-shaped set, lying on the concave side of the two rows, to which it gives off fibres along its whole course. Before this division of the nerve takes place, a few large fibres run out from it on the lateral side (Fig. 40) to supply the short transverse row of large bristles (Plate 7, Fig. 33).

The sensory nerve cells lie immediately beneath the hypodermis, and their peripheral fibres run in a plane parallel with the floor of the sac. In the case of the transverse row of large hairs, the nerve cells are situated about 450 μ posterior to the bases of the shafts, their peripheral fibres being therefore nearly half a millimetre in length. This is accounted for by the position of the new hair tube during the period of its formation between moults, when it extends back from the base of the functional shaft 350 μ; the distance from base of hair to ganglion cell must consequently be somewhat greater than this.

a. Number of Nerve Elements to a Single Bristle. The number of cells and fibres for the whole sac could not be determined with exactness, as other sensory elements, supplying tactile hairs, are mingled with those of the otocyst. But in the short transverse row of large hairs, the cells and fibres are sufficiently isolated to allow of their being counted in serial sections. There are but nine hairs in that row, and if the nerve elements supplying them were twice as numerous, it would be at once apparent. The cells always occur singly, and their fibres run separately and parallel with one another to the bases of their respective hairs (Plate 7, Fig. 33). The number of each was counted many times, and it is certain that *the number of ganglion cells and peripheral fibres exactly equals the number of hairs.* Whole preparations of these nerve elements stained with methylen blue gave regularly nine ganglion cells and fibres supplying the nine sensory hairs. In these few otocyst hairs, at least, there is, then, but a single nerve element supplying each.

In the *tactile hairs* of the scaphognathite of the second maxilla, many methylen-blue impregnations gave conditions like that shown in Figure 34 (Plate 7), only one sensory nerve element being stained. In the short spike-shaped bristles found on this same appendage, from three to five ganglion cells (Plate 7, Fig. 32, *cl. gn.*) were usually found supplying each bristle.

In the *olfactory bristles* of the antennule, the conditions were the same as those already described and figured for Palæmonetes, though fewer elements compose each spindle-shaped group of cells.

b. Peripheral Terminations. No branching of peripheral nerve fibres was observed in any sensory elements, though many were traced the whole length of an appendage. In Cambarus the fibres end always at the base in the otocyst hairs (Plate 7, Fig. 33). There is often a marked increase in the diameter of the fibre near its termination, caused either by the staining of its sheath at this point, or by a partial separation of the component fibrillæ. *Tactile hairs* show similar conditions in their nerve endings (Plate 7, Fig. 34).

The fibre strands of the *olfactory bristles* were, on the contrary, traced into the shaft some distance, where they apparently end free. Thus in the crayfish, we have a distinct difference in the innervation of the two types of sensory hairs, which serves to confirm the statements made concerning the conditions in Palæmonetes and Crangon.

c. Central Terminations. The otocyst nerve in Cambarus is large enough to be dissected out and traced to the ventral side of the brain, which it enters lateral to the larger antennular nerve. Its point of entrance is a little to one side of the median plane of the brain, opposite the posterior end of the globulus (Plate 9, Fig. 41). Its fibres run backward and dorsad, just lateral to those of the antennular nerve, and end in a neuropil directly anterior and median to that of the second antenna (Fig. 41, *n. ot.*). The individual fibres end by branching into fine fibrillations, which could be traced only a short distance through the diffusely stained mass of fibrillar tissue about them.

d. Histology of the Nerve Elements. The sensory nerve fibres of Cambarus are relatively smaller than those of Palæmonetes. Immediately after leaving the ganglion cell each measures about 3 μ in diameter, but becomes smaller as it runs distally, until near the point of ending, where it again enlarges to its original size. In well differentiated methylen-blue stains, fibrillar structure is clearly brought out. Longitudinal sections, and whole preparations of continuous fibres, show fibrillations similar to those figured for Palæmonetes.

The sensory nerve cells are relatively large; they measure from 15 to 18 μ in diameter, and being bipolar are spindle-like in form. Their nuclei are spherical and from 10 μ to 12 μ in diameter. The cytoplasm of the cell never shows any evidence of fibrillations, but in methylen-blue impregnations there is a faintly staining zone directly about the nucleus; the remainder of the cytoplasm takes on a deep blue color. This difference in staining qualities may be due to the unequal distribution of chromatic substance in the cytoplasm.

The myelin sheath, so characteristic for the nerve fibres of Palæmonetes and Crangon, is not found in the nerve elements of the crayfish.

3. *The development of the otocyst* was not studied in Cambarus. According to Reichenbach ('86) it is completely formed before the young animal leaves the egg.

IV. CARCINUS MÆNAS LEACH. (*Green crab.*)

We now come to the second type of otocyst, which is found in all brachyuran Crustacea; it is closed, and without otoliths. Mistaken by Bate ('58) for an olfactory organ, and figured by him in the larval stages of the crab, it has been described carefully in Carcinas mænas by Hensen ('63) alone. His account, although fairly accurate, is influenced by his seeing a fancied resemblance between the otocyst and the vertebrate ear; the figures he gives of different parts of the sac dissected out leave one somewhat in the dark as to the relative positions of the structures described.

1. *Structure of the Otocyst.*

a. Sac. The basal segment of the antennule in Carcinus is relatively large, and elongated laterally to such an extent that its width is nearly twice its length (Plate **9**, Fig. 46). Along its dorsal wall there extends transversely a distinct line dividing the chitin of the anterior part of the segment (*lab. a.*) from that of the posterior. This line of division, which reaches from the lateral margin of the segment three-fourths of the way across its dorsal wall, is rendered more prominent from the fact that the chitin posterior to it (*lab. p.*) is much lighter in color than that in front.

If the antennule of a crab is examined directly after ecdysis, when the chitin is still very thin, soft, and uncalcified, this lighter colored area (Fig. 46, *lab. p.*) is found to be a fold, projecting forward over the anterior part; and if its edge is lifted with a needle or fine pair of forceps, a transverse aperture is disclosed leading down to the lumen of the sac. This aperture extends from line 45 (Fig. 46) laterally down through the side wall of the antennule. There is, then, in fact, a free passage into the otocyst directly after moulting, a condition necessitated by the casting off of the old sac. But almost immediately after ecdysis, the opening is closed and its edges fuse together, probably owing to the simultaneous secretion of chitin by the hypodermis of the two surfaces which bound the orifice and are in direct contact. Figure 44 (Plate **9**) shows at *lab. p.* the two surfaces which fuse.

The form of the sac is very irregular, so much so that Hensen despaired of describing it. Its walls, like those of the forms already studied, are continuous dorsally with the calcified chitin of the antennule (Figs. 42–48, Plate **9**). The sac is thus suspended from the dorsal wall of the appendage. Although composed largely of thin chitin, one portion of its wall is much thickened and calcified (*mal.*, Figs. 43–48, Plate **9**). On account of its irregular outline measurements can be of only small value. The average of a number of measurements taken of the otocyst in specimens approximating 30 mm. in length, gave the following results: —

Greatest length, 1.11 mm.
" width, 1.96 "
" depth, 1.05 "

The seemingly contorted shape of the sac is caused by three protuberances or invaginations of its walls, which project into the lumen (Fig. *A*, and Plate **10**, Fig. 55). Two only of these prominences are sensory and bear bristles (Fig. *A*, *set. ta.* and *set. fil.*). The third and largest of the three (*mal.*), which projects from the lateral and posterior wall of the cyst, is without sensory organs of any kind. Its wall is irregularly curved and pitted (Plate 9, Fig. 47 *mal.*,), while portions of it are even calcified. At one point its walls are constricted to form a neck, which bears a large hammer-like head (Fig. 47). This is the "Hammer" of Hensen, compared by him to the malleus of the vertebrate middle ear. Figures 43, 48, and *A* show the relative position of this hammer to the rest of the sac. It serves merely for the attachment of the short, thick, powerful muscles of the antennule which keep the latter in almost constant motion, and has probably nothing whatever to do with the sensory functions of the otocyst.

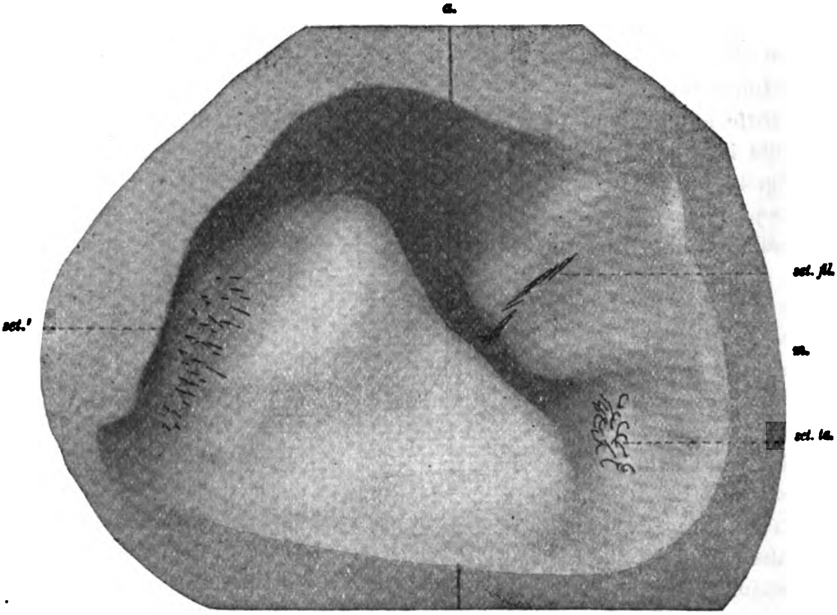

FIGURE *A*.

Model of the lumen of the left otocyst of Carcinus, dorsal view, the upper wall of the sac removed. The cavity of the sac was modelled in wax from serial sections under a magnification of 50 diameters, and a plaster cast of the model photographed natural size. In making the cut this was reduced to a magnification of 33 diameters. *a.*, anterior; *m.*, median; *set.'*, group hairs; *set. fil.*, thread hairs; *set. ta.*, hook hairs.

b. Sensory Cushions. Of the three projections noted, the remaining two are sensory and bear sensory hairs (Plate 10, Fig. 55, *set. ta.*, *set. fil.*). The smaller of these (*set. ta.*), located on the median portion of the posterior wall of the sac, bears a number of hairs with hooked shafts. The surface bearing these lies in a nearly vertical plane. From its position and the shape of its hairs this prominence is comparable to the sensory cushions upon the surfaces of which the otoliths are lodged in Palæmonetes, Crangon, and the crayfish. Irregularly disposed matrix cells are situated in clusters immediately beneath the hooked hairs (Plate 10, Fig. 50), and deeper in the tissues are the ganglion cells of the nerve fibres which supply the bristles (Fig. 50, *cl. gn.*). In the larval stages of the crab this sensory cushion is relatively much larger. It extends through half the length of the sac, and its hairs are in contact with the otoliths which the sac then contains. Pores of tegumental glands penetrate the chitin of this prominence, as they do that of the sensory cushions in the crayfish and lobster, although found in no other part of the sac. These glands secrete a substance which, in the larval crab, attaches the otoliths to the tips of the hairs. Their presence in the adult crab is evidence in favor of the homology of this cushion with that described for otocysts containing otoliths.

The other sensory cushion is much larger, and is produced by a partial invagination of a portion of the median and anterior walls of the sac, which forms an oval prominence (Fig. *A;* Plate 9, Fig. 48; Plate 10, Fig. 55, *set. fil.*). It is nearly 0.5 mm. in diameter, and its surface, making an angle of about 45 degrees with both the transverse and sagittal planes of the animal, inclines backward, inward and downward (Plate 9, Fig. 45). Its ventral portion is shown in transverse section in Figs. 47 48. The chitin of this cushion is very thin; upon it is a row of long delicate hairs, called by Hensen ('63) "Fadenhaare," or thread-hairs. This row runs down somewhat obliquely from the upper side of the prominence to its ventral margin near the floor of the sac, its dorsal end being the more anterior of the two (Fig. *A*, *set. fil.*).

This sensory cushion is also found in the sac of the larva, and the bristles it then bears are similar to those found projecting free into the lumen of the lobster otocyst from its median wall (Plate 5, Fig. 26, *set. m.*). The prominence we are now describing in Carcinus is probably therefore simply a further differentiation of the slight projection noted in the sac of the lobster.

Matrix cells send delicate processes into the hairs, as in those of preceding species; the ganglion cells are situated directly beneath the hypodermis, but some distance posterior to the bases of the hairs (Plate 10, Fig. 53, *cl. gn.*).

No gland pores are present, nor are they needed, as the thread hairs are never in contact with the otoliths, even in the larval stages.

A third region, on which sensory hairs are located, is found at the extreme lateral side of the sac, beneath the fused lips of its opening (Fig. *A*; Plate **9**, Fig. 42, *set.'*). There is only a slight prominence, the surface bearing the hairs being nearly flat. The hairs are arranged in irregular fashion, somewhat like the groups of otocyst bristles situated near the aperture of the sac in the crayfish and lobster. Numerous groups of matrix cells lie directly below these hairs, but no nervous structures could be distinguished in their vicinity.

The great hammer-like prominence, which serves for the attachment of the antennular muscles, separates the sac roughly into an upper, anterior chamber and a lower, posterior one. The first of these compartments is again partially separated into two by the anterior sensory prominence, which nearly meets the "hammer." These three chambers, into each of which sensory hairs project, were likened by Hensen to the semi-circular canals of the vertebrate ear, and the sensory regions to the *cristæ acusticæ*. As the compartments are in free communication, are not at all canal-like in form, and are arranged in no definite positions relative to each other which might be of functional importance, there seems to be no more logical reason for making such a comparison than for comparing the hammer-like projection of the otocyst to the malleus. The apparent division of the otocyst into three compartments is not a modification for the purpose of increasing its usefulness as a sense organ, but evidently a condition brought about mechanically by the differentiation of the "hammer" along lines which would make it better adapted for the attachment of muscles.

c. Structure of Hairs. The hairs, as already indicated in describing the sensory regions, are of three kinds. Hensen's account of them is fairly good. He divides them into the following classes: (1) hook hairs (Hakenhaare), (2) thread hairs (Fadenhaare), and (3) grouped hairs (Gruppenhaare).

(1) *The hook hairs* are found on the posterior vertical cushion (Fig. *A* and Plate **10**, Figs. 50, 55, *set. ta.*) arranged in a very irregular curved row. They vary from 25 to 31 in number, and are relatively very small, averaging 49 μ in length and 4 μ in diameter. Their shafts are hooked, often bent nearly double, and are sparsely fringed near the tip, if at all. The base is enlarged, as is usual in otocyst hairs, but not so markedly as in the forms already studied. Instead of being attached to a large spherical membrane, the base of the shaft is set

into a cup-shaped depression and so labilely fastened to the chitin of the sac wall (Plate 10, Fig. 51) that the hair can sway freely in any direction, as if it were attached by a ball-and-socket joint. This cup-like depression is characteristic of all the otocyst hairs of Brachyura.

The hook hairs are present in the otocyst of the Megalops larva of Carcinus, and are there relatively much larger; they extend over a large portion of the posterior end and floor of the sac, the curved row of 25 to 30 hairs occupying two-thirds of its length. As the otocyst is open at this stage, it contains numerous otoliths, and these are either *in contact with, or attached to, the tips of these hairs.* Measurements of a number of these larval hairs were made in the Megalops and the stage succeeding it, and a comparison of these with the same hairs of adults is made in the following table:

STAGE.	Width of Otocyst.	Average Length of ten Hook Hairs.	Average Diameter of ten Hook Hairs.
Adult (80 cm. long)	1.96 mm.	49 μ	4 μ
Young crab	0.24 mm.	47 μ	4 μ
Megalops larva	0.21 mm.	46 μ	4 μ

This table brings out the interesting fact that the hook hairs of a Megalops larva, of a young crab and of an adult are of nearly equal size, although the otocyst of the adult is nearly ten times as long as that of the Megalops, and over eight times that of the young crab. On measuring the thread hairs to see if the conditions there were the same, it was found that in the adult they were three and a half times as long as in the Megalops stage; the thread hairs thus more than tripled their length, while the hook hairs remained constant. The number of hook hairs is approximately the same in the Megalops otocyst and in the sac of the adult. Their arrested development may be explained by the fact that they are true otolith hairs; when the otocyst becomes permanently closed, otoliths can no longer enter the sac, and these hairs, as they lose their original function, do not grow *pari passu* with the other hairs of the otocyst, but remain unchanged. They do not degenerate and become entirely functionless, for they are still innervated in the adult crab, and, though sac after sac is shed and new ones formed without an otolith's finding its way into the organ, they still retain the peculiar form of the original otolith bristles.

We are thus led to regard the hook hairs of the crab as homologues of the otolith hairs of Macrura, and for these five reasons:— (1) The similarity in their structure. (2) Their similarity in position at the posterior end of the sac. (3) Otoliths are in contact with the hook hairs in larval stages, though not in the adult. (4) When the otoliths disappear, the development of the hook hairs is arrested. (5) Gland pores open through the chitin of their cushion, as they do through that of the crayfish and lobster, although they are not found in the other sensory regions of the sac.

(2) *The thread hairs* are the largest, the most highly differentiated, and probably the most active sensory bristles of the otocyst. There are about thirty of them, arranged upon the large anterior sensory cushion in a regular row (Fig. *A*, *set. fil.*). These hairs are extremely attenuate. Measuring only two or three μ at the base, the straight or slightly bending shaft averages $320\,\mu$ in length; it is unfringed save at the very tip, where for a short distance it bears two rows of extremely delicate pinnules. A peculiarity of this fringed tip is that it is not a continuation of the main shaft of the hair, but seemingly a diminutive hair in itself, sprouting from the latter. It makes a slight angle with the main shaft, the end of which projects a short distance beyond the base of the offshoot (Plate 10, Figs. 53, 54). Tho shafts of these hairs are directed out laterally, and slightly posteriorly, into the fluid contents of the sac, and they are so delicately attached at their bases that the slightest jar imparted to the liquid in which they float is sufficient to set them swaying. In alcoholic material they break off very easily. The shaft decreases somewhat in diameter towards its base and then suddenly enlarges. This enlargement is attached to the floor of a deep cup-like socket, the orifice of which is large enough to give ample play to the shaft in its movements (Fig. 53).

Straight attenuate hairs are found in the otocyst of the Megalops larva having the same relative position in the sac as the thread hairs of the adult. These hairs are not in contact with otoliths, but each shaft is fringed with filaments throughout its whole length. They become differentiated in later stages into the peculiarly modified thread hairs. Hairs similar to those of the Megalops larva just described are also found in the otocyst of the adult lobster, situated on the median wall of the sac and projecting free into its lumen. They are similar in both larva and adult, and are probably in function accessory to the otolith hairs. They may be homologues of the thread hairs, which, in the crab, with the disappearance of the otoliths, have taken on the chief functional activity of the otocyst, formerly vested in the hook hairs.

(3) *The group hairs* (*set.'*) form the third and most numerous class of the otocyst bristles of Carcinus. Irregularly distributed in the most lateral corner of the sac (Fig. *A*,) on a flattened portion of the wall ventral to the closed margins of the aperture (Plate 9, Figs. 42, 47; Plate 10, Fig. 55), they are unlike any of the otocyst hairs found in Macrura, being short, thick, and blunt, without a trace of fringing filaments (Plate 10, Fig. 49). They are 110 μ to 135 μ long and 12 μ to 14 μ in diameter. There are nearly 200 of these hairs, forming one large irregular group. They do not occur in the Megalops otocyst, therefore they must be developed at some later period. They may possibly be degenerated tactile hairs which in the formation of the otocyst have been folded into its cavity. Their proximity to the aperture of the otocyst makes this supposition highly probable. Their shafts are set into depressions in the sac wall, and, like the other otocyst hairs, they can sway freely on their bases.

d. Formation of Hairs. The hairs are formed in Carcinus, and in the Brachyura generally, after the method already described in Palæmonetes. From the presence of a cup-like depression at the base of each shaft, instead of the large spherical membrane found in the Macrura, it might be inferred that the cup results from the incomplete evagination of the hair.

e. Otoliths are entirely wanting in the adult otocyst, but are present in those larval stages where the sac is still open. They consist, as usual, of grains of sand, which in this case are very small, for the sac itself in these stages is less than 0.3 mm. in length. They can readily be introduced into the otocyst of the Megalops, as its aperture is relatively large. When in a succeeding stage the sac is cast off with its otoliths at ecdysis, the aperture of the new cyst closes at once, and no foreign particles can enter it; henceforth it is without otoliths.

2. *Innervation of the Otocyst.*

The general course of the otocyst nerve is shown in Plate 10, Figure 55 (*n. ot.*). As in the forms previously described, the sac lies in close proximity to the brain, and its nerve is consequently short. It is given off with the antennular nerve from the anterior end of the central organ, and its course for a short distance is directly lateral, until the base of the antennule is reached. At this point the antennular nerve (*n. at.₁*) turns straight forward, while that of the otocyst divides into three branches (Fig. 55, *n. ot.*, *n. ot.′*, *n. ot.″*). The most median and largest of these runs forward to supply the thread hairs; the middle branch goes directly to the posterior sensory cushion, which bears the hook hairs; while the third and lateral offshoot takes a nearly straight course along the posterior wall of the sac and supplies the tactile hairs of the antennule, and possibly the group hairs of the otocyst. The ganglion cells of the hook hairs are some distance posterior to the hairs and arranged in an irregular scattering group (Plate **10**, Fig. 50, *cl. gn.*). Those of the thread hairs are lateral and posterior with reference to their hairs, lying immediately beneath the hypodermal cells of the sensory cushion, and forming an irregular single row, which is nearly parallel to the row of thread hairs (Plate **10**, Fig. 53, *cl. gn.*).

a. Number of Nerve Elements to a Single Bristle. The nerve elements of the thread hairs were brought out clearly and completely by methylen blue and by Vom Rath's platinic-chloride method. The conditions found in a number of preparations are shown in Figure 53, where there is but a single element for each hair. This particular preparation was obtained with methylen blue, but the results were verified by Vom Rath's method. Counted in serial sections, the number of hairs and ganglion cells were approximately equal.

By the same method of counting, the elements of the hook hairs gave like results. In one case there were thirty hairs and thirty-one cells. No ganglion cells could be made out near the group hairs, nor any fibres supplying them. Certain clusters of cells are found directly beneath their bases, but their large peripheral processes, irregular outlines, and lack of central fibres marked these as matrix rather than nerve cells.

Here in Carcinus, then, as in the macruran forms described, *there is but one nerve element to each otocyst hair.*

The distal segment of the antennule was by chance sectioned in making preparations of the otocyst, and when stained with iron hæmatoxylin, the innervation of the *olfactory* hairs found in that region was sharply brought out (Plate **10**, Fig. 52). As in the examples of this type of hair already described, a large spindle-shaped group of about 100 ganglion cells sends a strand of nerve fibres to the base of each shaft. These cells are relatively small and situated 0.5 mm. posterior to the hairs they supply. In Figure 52 a single nerve element is shown diagrammatically in black.

b. Peripheral Terminations. As seen in Figure 53 (Plate **10**), the terminal fibres going to the thread hairs enter the pore at the base of the cup-shaped depression, pass up into the enlargement of the hair shaft, and there end free. In fact, there is in these hairs no functional necessity for the further continuance of the fibre into the shaft. Since the hairs project free into the liquid of the sac, if the otocyst is jarred or tilted, the shaft does not itself bend, but sways backward and forward upon its base. It is therefore at the base that the stimulus must manifest itself, and it was there in every case that the fibres were found to end.

In the olfactory hairs, on the other hand, the nerve fibres continue up into the large hollow shafts for some distance (Plate **10**, Fig. 52, *set. olf.*). The olfactory hairs of Carcinus thus differ in their innervation from those of the otocyst, both *in the number of nerve elements* supplying each hair, and *in the peripheral nerve endings.* In the bristles of the otocyst there is but a single nerve element, and it ends free at the base of the hair without branching. In the olfactory hairs there may be a hundred elements or more which end in the shaft of a single hair.

c. Central Terminations. Entering the brain in front of, and just median to, the globulus, and ventral to the optic centres, the fibres of the otocyst nerve run straight back and enter the fibrillar mass (Plate **10**, Fig. 55, *n'pil. at.1*), called "the neuropil of the first antenna" by Bethe ('97), who has described the central endings of the antennular nerve of Carcinus. The fibres of the antennular nerve end in a connected neuropil just median to those of the otocyst. Bethe judged from his physiological experiments that there should be certain fibres from the otocyst ending in the globulus. He was not able to demonstrate such endings with methylen blue, nor was there any evidence of their existence in my preparations. According to Bethe the fibres from the otocyst end by the separation of their fibrillæ in the neuropil. Lack of fresh Carcinas material prevented the verification of his work, but I have described similar conditions in the shrimp and crayfish.

d. Histology of the Nerve Elements. As the finer structure of the elements of the central nervous system has been fully described by Bethe ('98), it is unnecessary for me to say anything on that matter, and only a few words need be added here as to the histology of the peripheral nerves and cells. The peripheral nerve fibres are much smaller than in Palæmonetes or Crangon, and are without a myelin sheath. The peripheral ganglion cells are relatively large, averaging 12 μ in diameter. They are of the typical bipolar form, and are much elongated (Plate

10, Fig. 50, *cl. gn.*). Their nuclei are nearly spherical, and contain at least one large deeply staining nucleolus. No special preparations were made for the purpose of demonstrating fibrillæ in either nerve cells or fibres. Bethe found them in all fibres, and traces of them in the cells of the brain.

3. *Development of the Otocyst.*

For the purpose of comparison with development in the lobster, the antennules of the first five free swimming larval stages of Carcinus were dissected out, stained and examined *in toto*. By this means it was ascertained that *there is no functional otocyst in the Zoea stages.*

(*a*) *The first Zoea* shows no trace of invagination in its antennule. There is, however, an aggregation of nuclei beneath the chitin of the region where the otocyst is to appear.

(*b*) *The second Zoea* shows a slight depression on the dorsal side of the antennule, and its basal portion has begun to widen.

(*c*) *In the third Zoea* this widening has increased, and the lateral wall of the antennule has now formed a rounded protuberance. The invagination has increased in size and depth, but no hairs nor otoliths are yet contained in it.

(*d*) At *the Megalops stage* we find that a sudden development has taken place, as in the fourth larval stage of the lobster. The Zoea has by a single moult become metamorphosed into a Megalops, and the otocyst changed from a shallow depression to a nearly closed sac, containing sensory hairs and otoliths. Two sensory cushions are present: one of these, posterior and median, bears 25 to 30 hooked hairs, upon the tips of which otoliths rest; the other prominence projects from the anterior portion of the median wall, and bears a vertical row of about 30 hairs, the shafts of which are directed laterally. These hairs are long, attenuate, and well fringed with delicate filaments. They do not come into contact with the otoliths, and, as already noted, they develop into the thread hairs of the adult; those of the first sensory cushion described correspond to the hook hairs of the mature crab. The third type of hair found in the adult is not developed at this stage. The aperture is anterior and lateral in position, and extends transversely across the antennule.

(*e*) The next stage examined was that of a young crab probably of the stage immediately succeeding the Megalops larva. The otocyst is slightly larger, and its opening is already nearly closed. As a result, only a few small otoliths were contained in it.

The otocyst of Carcinus thus resembles very closely in its development that of the lobster. In both there is no trace of the organ in the newly hatched larvæ, and for three successive moults it is not functional. In the fourth larval stage, with a sudden metamorphosis of the animal's general form, the otocyst is also rapidly changed from a mere depression to an active, well-developed organ. The significance of these sudden correlated transitions will be seen when the otocyst is considered physiologically.

C. THEORETICAL CONSIDERATIONS.

1. Comparison of the Otocyst with the Vertebrate Ear.

The otocyst has been compared by many investigators to the auditory organ of vertebrates. Leaving their functions entirely out of account, how far do the two correspond in structure?

The otocyst of Macrura consists of an open sac, a sensory prominence, bristles, and otoliths resting upon them; essentially the same conditions as are found in the ear of Myxine, though the latter has five sensory regions instead of one. The otocyst of macruran decapods might thus be well compared to an isolated ampulla in the ear sac of Myxine, and the sensory cushion to a single crista acustica.

In the Brachyura the organ is still more highly differentiated. The sac is closed, there are three sensory regions, and the hairs found on them project free into the lumen of the otocyst; otoliths are entirely wanting. The structure of the sensory apparatus is in this case similar to that of the cristæ of higher vertebrates, and the sac itself resembles the utriculus. But *there is no portion of the decapod otocyst so differentiated as to bear more than a fancied resemblance to the semicircular canals, the middle ear, or the cochlea of higher vertebrates.*

Each crista acustica in vertebrates, however, is made up of separate elements, which may be compared to the sensory elements of the otocyst. Every auditory hair of the crista is developed from the exposed end of a specialized epithelial sense cell, which itself forms the basal part of the hair, and is supported in position by the other cells of the epithelium. It has been shown by both Retzius ('94) and Morrill ('98) that these epithelial sense cells of the cristæ in vertebrates are not true nervous elements, as the auditory fibres are not continuous with them. Both the cell and its auditory hair taken together are to be compared to the bristles of the otocyst, in that they constitute a non-nervous end-organ.

Their innervation is also essentially the same. In the vertebrate crista an auditory nerve fibre passing from the brain is connected with a bipolar nerve cell in the auditory ganglion, from whence its peripheral fibre extends to one of the epithelial sense cells, ending with a slight enlargement in close proximity to, or in contact with its base. *The single fibre supplying each end-organ is never directly connected with the cell, nor does it ever run through it to the hair itself.* The only difference between the peripheral endings just described, and those of the otocyst, is that in the hairs of the latter the fibres end free in the base of the hollow shaft, at the point where, from the structure of the hair, the greatest stimulus would be produced; while in the vertebrate end-organ the nerve process is applied to the convex under-surface of the basal cell, which would transmit stimuli with an equal degree of intensity to fibres in contact with it at any point.

The otoliths of the vertebrate ear are formed by secretion, while those of the crustacean otocyst are largely granules of sand taken into the sac from the exterior. In some Crustacea, however, such as the Mysidæ, and in many other invertebrates, the otoliths are formed within the sac.

In all decapods the innervation of the otocyst hairs distinctly differs from that of the olfactory bristles, not only as to peripheral terminations, but also in the number of nerve elements supplying each hair. As has been previously noted, the stimulus is transmitted by specialized cells or hairs to the nerve fibres of both the otocyst and the vertebrate ear, and is never applied *directly* to their endings. In either case *only one nerve element* is usually in contact with the terminal sense cell, and this is apparently ample to carry the isolated nervous message to the brain.

With the olfactory sense it is different; in both vertebrates and Crustacea the chemical stimuli which produce the olfactory sensations act directly upon the nerve cells or their terminal fibres. In vertebrates portions of the nerve cells are exposed at the surface of the olfactory epithelium. In crustacea peripheral fibres from the ganglion cells of the olfactory nerve end free in the hollow, perforate bristles. In Nereis and the earth-worm, Langdon ('95, '00) has shown that the processes of the olfactory cells end free upon the surface of the cuticula, and completely exposed to chemical stimuli; a similar condition has been shown by Lewis ('98) to exist in two polychætous worms of the family Maldanidæ.

The large numbers of nerve elements ending in each olfactory tube or bristle of decapod Crustacea may be accounted for by the fact that the stimulating chemical substances occur as slight traces only. In order that a sensation may be perceptible, apparently a large number of olfactory elements must be stimulated at once, for the larger their number, the stronger should be the sensation produced. The olfactory bristles are located on the flagella of the antennules, a position most favorable for the reception of chemical stimuli, as the flagellum projects some distance in front of the animal and can be kept in constant motion. The number of the bristles is limited on account of the small surface to which they are necessarily confined, so that, if thousands of olfactory fibres are to function simultaneously, large numbers of them must be exposed to the chemical stimulus in the same hair. It is possible, too, that different nerve elements may be affected by different substances in solution; and that consequently many olfactory elements are necessary for each hair, in order that different chemical stimuli may be perceived.

2. The Neuron Theory.

The conditions found in the sensory nerve elements of the otocyst are favorable to the neuron theory, in so far as they confirm the generally accepted idea that the nerve fibres are each differentiated from a single nerve cell, and that fibre and cell taken together form a trophic unit. This conclusion is borne out not only by the structural conditions already described, where each fibre is connected with only one peripheral ganglion cell, but also by an experiment which I made by severing the otocyst nerve proximal to its ganglion; in this case after the lapse of a few weeks degeneration of the sensory fibres took place back into the brain.

As to the modifications of the neuron theory recently proposed by Apáthy ('97) and Bethe ('98), — that the neurons are connected by fibrillæ, — the fibrillar structure of the fibres is confirmed by my preparations, though no fibrillæ could be demonstrated in the nerve cells. In regard to the definite connection of the neurons with each other by continuous fibrils, such as Apáthy figures and describes in the Hirudineæ, my preparations gave no positive evidence; but the fact that the central fibrillations of the nerve elements of the otocyst could not be traced to determinate endings, makes it quite possible that such a direct communication between motor and sensory neurons may exist. While Bethe proved that there were more fibrillæ in a motor fibre than extended into its central ganglion cell, and also, that some fibrillæ entered the fibre by one branch and at once passed out by another, in no case did he trace a single fibril from one neuron into another. If such a connectio between nerve elements had been demonstrated beyond a doubt, they might still be considered as distinct trophic units, and the interdigitating fibrils uniting them as the products of separate neuron cells. In the light of the important discoveries of Apáthy and Bethe, however, the old view, that the nervous impulses are transmitted from sensory to moto neurons by the simple contiguity of their dendritic processes, may hav to be abandoned for the more reasonable assumption of direct fibrilla communication.

PART II. — PHYSIOLOGY.

As Bethe has well said, the best of anatomical knowledge concerning an organ cannot be taken as certain evidence of its functions. It is only after these functions have been experimentally demonstrated, that we may ascribe them with confidence to the organ in question.

Have we, then, any experimental proofs that the decapod Crustacea hear? If so, is the otocyst the auditory organ; if not, what is its function? These are the three chief questions which I shall attempt to answer.

A. HISTORICAL SURVEY.

Up to the time of Delage (**'87**) the auditory function of the otocyst was accepted, and that alone.

Minasi (**1775**) promulgated the idea that Crustacea could hear. The hermit crab, Pagurus, was more sensitive than man to sound vibrations. The tones of a distant bell, the striking of a clock, were, according to this worthy monk, perceived by Pagurus sooner than they were by him.

Ælianus (**1784**) notes that the fishermen of his time took Pagurus by means of music.

All the older zoölogists have regarded the otocyst as an organ of audition.

Hensen (**'63**) was the first to get experimental data. From the anatomical conditions found in the otocyst of the lobster, he argues as follows: Here are 468 auditory hairs upon which otoliths rest. Of these hairs no two are of the same size; they vary in a nearly continuous series from 0.72 mm. to 0.14 mm. in length; thus the volume of the largest is to that of the smallest as 140 : 1. Comparing these ratios to those of the volume of organ pipes, we should have, *if the hairs responded to different sound vibrations,* an auditory organ with a range of three octaves.

To prove that his hypothesis was correct, sound waves were conducted, by a mechanical contrivance modelled after the middle ear of mammals, into the water of a vessel containing Mysis, the so-called auditory hairs of which were under observation by the microscope. When notes of a certain group were sounded on a musical instrument, a certain hair would vibrate and disappear from view. Others would also respond, but each to different sets of notes.

Having proved that the different hairs responded to different sound waves, Hensen next determined that Crustacea would react to vibratory stimuli. A resonant bar of wood was floated in a vessel containing free-swimming individuals of the genera Mysis and Palæmon. When the bar was struck, both forms responded by a strong leap away from the source of the sound. Palæmon reacted even more strongly when rendered sensitive by gradual strychnine poisoning.

Milne-Edwards (**'76**), Jourdain (**'80**), Delage (**'87**), and many others have accepted the sense of audition in Crustacea as a fact.

Garbini (**'80**, p. 192) uncritically remarks: "Che i crostacei odano è indubitato; lo sanno anche i pescatori, i quali devono avvicinarsi loro in silenzio" (That crustacea hear is undoubted; this the fishermen know well, who, when they capture them, approach in silence).

Individuals of Palæmonetes varians, which he kept in an aquarium, sprang backward at the slightest sound.

Delage (**'87**) was the first to discover another function than that of audition for the otocyst. By cutting off or destroying the sacs, he proved that they functioned also as organs of orientation. Animals so operated upon (Mysis, Palæmon, and Polybius among Crustacea) were unable to keep their normal upright position in swimming. Blinding intensified the effect, showing that sight aided in orientation.

The otocyst may therefore, in his opinion, be compared to the simplest form of the vertebrate ear, — that found in Myxime, — where the semicircular canals and utriculus serve the purpose of orientation, the sacculus that of audition (to intensity of sound). In the otocyst of Crustacea both functions are performed, he believes, by the same organ.

Verworn (**'91**) proved that the otocyst of Ctenophores served simply for orientation, not being sensitive to sounds.

Bunting (**'93**) confirms the conclusions of Delage as to the function of the otocyst in geotropic orientation. When the otocysts of young crayfish were destroyed, especially if their chelæ were also removed to render their position in the water less stable, there was the same loss of power of orientation that had been observed by Delage.

Kreidl ('93), in order to avoid the disturbance to the normal condition caused by the removal of the otocysts, made use of the following ingenious experiment: Palæmonetes newly moulted, and thus without otoliths, were placed in filtered water to which iron filings were added. The otocysts were soon filled with the metallic particles, the chelæ being used to convey them to the opening of the ear in the dorsal wall of the antennule. When now a strong electromagnet was held at one side of, and slightly above the sacs containing the iron otoliths, the shrimp would lean a little to one side, its dorso-ventral axis, normally coincident with the direction of gravity, pointing away from the magnet. This new position of the dorso-ventral axis is proved by mechanics to be the resultant of the two pulls, that of gravity and that of the magnet, the animal accommodating itself to the direction of the resultant of the two forces. If the magnet were held to the right of the animal, the otocysts would be stimulated in precisely the same way as by gravity alone when the shrimp's dorso-ventral axis is artificially turned toward the right; the result is that it attempts to recover its normal position with reference to gravity, and thus turns its vertical axis away from the magnet. Kreidl, going a step further than his predecessors, affirms that the otocysts are not auditory, but exclusively static in function. Thus they should be called *stato*-cysts, not *oto*-cysts.

Still further evidence as to their static function is supplied by Clark ('96). The compensation movements of the eyestalks of the fiddler crab (Gelasimus pugilator) and the lady crab (Platyonichus ocellatus) were observed. Tilting a normal animal about its antero-posterior axis gave a parallel compensating movement of the eyes through an angle of 35° to 45°, whether the tilting was to the right or left. On rotation about the dorso-ventral axis, no such movements are shown, though when rotated about the lateral axis, the animal's eyes moved in the opposite direction through an angle of 35°.

If both otocysts were removed, these compensative movements were much reduced, and the general movements of the crab also became very uncertain.

After removal of one otocyst 94 per cent of the animals showed on rotation toward the uninjured side less compensation than uninjured animals. Blinding produced only a slight reduction in the compensatory motions, but when, in addition to this, both otocysts were destroyed, compensatory movements completely disappeared.

Bethe (**'97**), in his physiological work on Carcinas mænas, confirms Clark's results. In a previous paper he (**'95ᵃ**) observes that *Mysis can hear after the otocysts have been destroyed*, but with difficulty; also that the animals are more sensitive to low tones than to high.

Thus, until 1898 three views were held as to the function of the otocysts:

(1) That they are purely auditory organs (Hensen and the earlier zoölogists).

(2) That they are both auditory and static in function (Delage and Bethe).

(3) That they are purely static in function, i. e. organs of orientation (Kreidl, Clark, and others).

To determine whether decapod Crustacea really hear, and if so, whether the otocyst is the organ of audition, is the aim of two papers by Beer (**'98**, **'99**).

In criticising the conclusions reached by Hensen and Bethe, Beer remarks in his first paper that, because decapods were made to react to different sounds, does not prove that these Crustacea responded to *true* sound, or that they heard. These reactions may have been due to their *feeling* vibrations transmitted to the water from the walls of the vessel in which they were confined, — a tactile reaction, or, to use Bethe's term, a "tango-reflex." Experiments with sounds produced in the air Beer considered superfluous, as it is a well-known physical fact that most of the sound waves are reflected from the surface of water.

Beer found that Crustacea reacted strongly to sounds produced in the water by striking partially submerged bells, jars, etc., but only when they were not at a greater distance from the source of sound than that at which vibrations could be detected by *the hand immersed in water.* The animals responded more strongly when near the walls of the vessel; but vibrations could be felt by the hand also in this position more distinctly, even though further removed from the source of the sound.

For animals well supplied with tactile organs, he regards pure sound or pure audition as impossible; because vibrations could be felt as soon as heard, and, this being the case, audition would be useless.

On removal of the otocysts, Palæmon and Palæmonetes still responded to sound waves produced in the water. There was, however, a slight inhibition of the customary reactions, therefore the hairs of the otocyst are probably slightly tactile as well as static in function.

From experiments on many different species of Crustacea, Beer ('98, p. 31) concludes: "Wir haben gute Gründe, dem in Rede stehenden Sinnesorgane der Krebse statische Functionen zuzuschreiben, und haben vorläufig gar keinen Anhaltspunkt, ihm Hörfunctionen, ja den Krebsen überhaupt Gehörsinn, zuzuschreiben."

Hensen's statement that the free auditory hairs of Mysis vibrated to different musical notes is simply an interesting physical fact. Hairs on the back of one's hand will do the same, but they are not auditory. The true sense of hearing is lacking not only in Crustacea, but probably in all other water-inhabiting animals lower than Amphibia, especially in invertebrates.

Beer thus comes back to the opinion of Johannes Müller ('37) expressed sixty years before: That in most invertebrates we find nothing comparable to the ear; and any reaction to sound vibrations should be attributed to a *tactile* rather than to an *auditory sense*.

A few months later Beer ('99) brought out a second paper, describing his experiments with blind shrimps, and answering a criticism of his previous work by Hensen ('99). Here the auditory sense, he urges, ought to be intensified, all possibility of sight entering as a factor into the experiments being effectually eliminated. The conclusions reached by him in his earlier work are verified in this.

General Criticism.

It is a noteworthy fact, that in the experimental work done to determine the function or functions of the otocyst, few of the investigators have acquainted themselves with the finer structure of the organ under consideration; one of the essentials for successful physiological work is a complete knowledge of the anatomical side of the subject. This is well illustrated in Bethe's work on the brain of Carcinas, where anatomical facts, obtained by means of methylen blue, laid the groundwork for his later confirmatory experiments.

Since the dissections by Hensen, little or no morphological work has been done on the otocysts of the Brachyura, yet a deal of physiological work has been attempted.

The experiments of Beer are beautifully worked out, and logical in sequence; yet, while he tried experiments on water-inhabiting animals, no attempt was made to experiment on amphibious decapod Crustacea, such as the fiddler crab. These animals, spending, as they do, a good share of their life on land, would certainly have more need of an auditory organ than decapods which are always beneath the surface of the water.

B. EXPERIMENTS AND OBSERVATIONS.

I. The Otocyst as an Auditory Organ.

That the responses of water-inhabiting animals to atmospheric sounds is nothing more than a myth, has been too well proved by Beer to need further investigation. The well-known physical fact that the larger part of the sound waves are reflected from a liquid surface is enough in itself to confute fables of fishes and crustacea hearing, and coming to be fed at the *sound* of a bell. But since in the case of responses of decapod Crustacea to sound vibrations conducted into the water, the experiments of Beer contradict Hensen's earlier results, repetition of Beer's work, though perhaps not absolutely necessary, may not be out of place.

METHODS.

The shrimps to be experimented upon (Palæmonetes) were placed in glass vessels 40 cm. in diameter and 20 cm. deep. Sound waves were conducted to the water by means of a steel pipe one inch in diameter and about two feet long, which was firmly clamped at its upper end and projected into the vessel containing the shrimps; a brass rod was in some cases substituted for the pipe. The pipe and rod were set into vibration either by striking them with a hammer, or by drawing across them, bowlike, a strap of rosined leather. Sounds were also produced by striking glass jars suspended in the water, and by striking the sides of the aquarium itself. The movements made in producing the sounds were completely screened from the view of the shrimps by pieces of cardboard placed over and at one side of the vessel, a small aperture being left for observing their reactions.

Palæmonetes could be made very sensitive to all nervous stimuli by leaving them over-night in sea water containing from 0.1 to 0.2% of sulphate of strychnia. This solution is fatal to a small fish (Fundulus) in five minutes; many of the shrimps die, but the sensory apparatus of those which remain alive is rendered abnormally acute. Blinding was accomplished by simply painting the eyestalks with a thick coat of lampblack and shellac; the otocysts were removed by means of a fine hooked needle, with scarcely any other injury to the animal.

1. *Responses of Palæmonetes to Vibrations transmitted to Water.*

a. Normal Conditions. Under normal conditions, when sound vibrations were transmitted to the water, normal animals responded by a slight leap backwards or to one side, if the source of the sound was within a distance of 20 cm. If an animal happened to be near the side of the vessel, and the sound was produced near the opposite wall 40 cm. distant, the response would be, *not a darting away from the source of the sound*, but a leap back from *the side of the vessel toward the source of the sound.* Again, if an animal was facing the side of the aquarium with its antennæ in close proximity to it, and the opposite wall was sharply tapped with the finger-nail, or lightly with a hammer, the shrimp, as before, sprang away from the side of the vessel *toward the source of the stimulus.* The response was usually well marked, a leap of from 10 to 15 cm. being made.

b. Poisoned with Strychnine. The responses obtained were invariably much stronger and more uniform with animals poisoned by strychnine in the manner stated above, than with normal shrimps. In other respects they were the same, and served merely to emphasize the results obtained by the first experiments. Blinded individuals showed practically the same reactions, but to make sure that the factor of vision was effectually cut out, the eyestalks of the shrimps in the succeeding experiments were all painted.

c. Both Otocysts removed. Of animals from which both otocysts had been removed, all but one gave a more or less strong response to the sounds conducted into the water in which they were swimming. The reactions were not as marked, nor could they be produced at as great a distance from the source of the sound, as in the case of normal animals. Nine individuals were affected by the stimulus when at a distance of about 10 cm.; the rest, only when in still closer proximity. A slight jar imparted to the walls of the aquarium produced essentially the same responses as the transmission of sound to the water by means of the vibrating pipe or rod. Removal of the otocysts has, therefore, only a very slight inhibitory effect upon the responses called forth by sound-wave stimuli in normal or strychnine-sensitized animals.

d. Removal of Antennæ and both Antennules. The removal of the antennæ and antennules, which bear large numbers of delicate *tactile* hairs, very much reduced the reaction of the shrimps to these vibratory stimuli. Only when an animal was in close proximity (5 cm. or less) to the source of the sound, or in contact with the walls of the vessel, would it respond, and then only feebly. Slight jarring of the aquarium produced no reaction, unless some part of the animal's body directly touched the sides or bottom of the jar, or was in contact with the sound-producing instrument.

The above experiments were duplicated on *Crangon vulgaris* with similar, though less marked results, as Crangon is much more sluggish than Palæmonetes.

A third set of experiments was tried with *Virbius zostericola*, a shrimp-like decapod *without otocysts*. Normal animals responded vigorously on striking a glass jar partially submerged beneath the water in which they swam. This response, much increased by strychnine poisoning, was distinctly diminished when both antennæ and antennules were removed.

e. Meaning of these Experiments. All of my experiments confirmed the conclusion of Beer, that free-swimming decapods, whether possessing otocysts or not, will respond to stimuli which are transmitted to them by the liquid medium they inhabit. The next question is, to determine whether this response is caused by the perception of *sound* waves or by the coarser vibrations or jars imparted to the water. In other words, have we to do with true *audition* or with the *sense of touch?*

Beer has clearly shown that there is no such thing as the transmission of pure sound waves from air to water. Coarser waves are imparted to the liquid simultaneously with those of sound, and can readily be felt by the immersed hand.

After making a number of trials with sounds produced as in the preceding experiments, I ascertained that the vibrations not only could be plainly felt by the submerged hand, but also that they could be felt *at a distance from* 10 *to* 20 *cm. greater than that at which the shrimps would react.* This fact does not at all prove that the animals experimented with do not hear, but merely shows that the responses supposedly produced by sound stimuli may be simple tactile reflexes, called forth by vibrations which, since appreciable to the immersed fingers, we may certainly assume to be *felt* by these animals, so well supplied with delicate tactile organs.

That the reaction is really due to tactile stimulus rather than to audition, is indicated by several facts brought out by the experiments:

(1) Animals, when near the wall of the vessel, even though distant from the source of the sound, respond vigorously, *leaping away from the wall and toward the sound.* The wall is set into vibration by the production of the sound, and it is apparently this vibration which affects them, rather than the true sound-waves imparted to the water.

(2) The average distance from the source of the sound at which they will respond is less than that at which vibrations may be felt by the hand.

(3) Removal of the antennæ and antennules which are supplied with numerous *tactile* bristles, inhibits the reaction.

(4) Decapods, such as Virbius, normally without otocysts *respond vigorously;* but removal of antennæ and antennules diminishes their sensibility in a marked degree.

(5) Precisely the same responses as were called forth by the production of sound were also obtained by simply tapping or jarring the walls of the aquarium.

Whether due to tactile stimulus or to audition, the fact remains, that the otocyst has little or no part in producing the reactions observed in the series of experiments; for (1) decapods normally without otocysts respond as vigorously to the same stimuli as those possessing them, and (2) the removal of the sacs from the latter has only a very slight inhibiting effect, which might be due either to the loss of these organs, or to the injury of the nerves supplying the many tactile bristles of the antennule.

Consequently, the otocyst not being the organ by stimulation of which responses to sound vibrations are called forth, and there being no other sensory apparatus in Crustacea especially differentiated for the reception of sound waves, we are led to the conclusion that in decapod Crustacea a true auditory organ is wanting.

The acute tactile sense of decapods may to some extent serve the same purpose that audition does in vertebrates. In mammals the senses of touch and hearing grade into each other. The range of the average auditory organ in mammals is from 30 to 16,000 vibrations per second; waves of less than 30 vibrations per second do not usually produce auditory sensations, but are appreciable to the tactile sense. It is important to note that decapods respond most vigorously to low notes, and not at all to high notes or sounds produced by very rapid vibrations. This fact would seem to be good evidence that the vibrations imparted to the water and perceived by decapods correspond to those which produce tactile rather than auditory sensations in vertebrates.

2. *Responses of Gelasimus pugilator* (*Brachyuran decapod*).

a. To Vibrations transmitted to Water. On the conduction of sound waves to water by the same means as in the preceding experiments, these fiddler crabs responded, but by no means as vigorously as did the Macrura. They always rested upon the bottom of the aquarium, and reacted by retiring slowly, either from the source of the sound, or from the vibrating walls of the aquarium. In either case the response took place only when the animal was within a few centimetres of the vibrating surface, and was most marked when the antennæ and antennules were in close proximity to it. After blinding the animals and removing their otocysts, no apparent difference could be detected in the reactions called forth, as compared with those of normal crabs; removal of the first two pairs of appendages caused, on the contrary, the responses to almost completely disappear.

b. To Atmospheric Sounds. As the fiddler crab is on land a large part of the time, a number of experiments were tried to determine the effect of aerial vibrations upon them when they were feeding under perfectly normal conditions. A position for observation was selected near a bank which was completely honeycombed by their burrows, where one could see the animals perfectly well, and yet be screened from their view by intervening bushes. If one remained perfectly motionless, the animals would come within a short distance of the observer's place of concealment, feeding as unconcernedly as if no one were near. When a number of crabs were little more than five feet distant, a horn was blown, care being taken to direct it away from them. Although a sound was thus produced loud enough to be heard at some distance, all the animals continued to feed undisturbed.

The striking together of two stones, and the sound produced by striking an iron pipe with a stone (the objects in both cases being held in the hand) also had no effect upon them. On striking the ground with a heavy stone all the crabs within a radius of ten or twelve feet were startled; some of them merely stopped feeding, while others scuttled into their burrows. The same result was brought about by simply stamping upon the ground. If a quick movement was made in the sight of the animals, they at once scattered precipitately to their holes. These observations were repeated a number of times, and on crabs of two different localities, with the same results.

From these experiments and observations, we may draw the conclusion that the fiddler crab, whether in water or on land, does not respond to true sound-stimuli, but is affected only by jars or vibrations transmitted to the water or to the ground. In neither case can they be said to hear. When feeding upon land they do not depend upon an auditory sense to protect them from terrestrial enemies, but rely entirely upon their keen vision and delicate tactile organs.

The statement is generally accepted, that all animals which produce sounds also have a sense of hearing, and this is advanced as an argument in favor of audition in Crustacea. The two well-known examples of sound production among decapods, observed by T. Parker ('78) and Goode ('78), are (1) the stridulation of the rock lobster, Palinurus, where the sound is produced by rubbing the second segment of the antenna against the antennule, and (2) the pistol-like report produced by Alpheus in snapping together the claws of the great chela. As Beer has pointed out, the otocyst is poorly developed in Palinurus; furthermore, no individuals of either species have ever been observed to respond in any way when these snappings or stridulations were produced.

We can no more argue, from these two instances of sound production in decapods, that there is an auditory function in all Crustacea than we can that all fish hear because the drum-fish makes a sound.

The enemies of water-inhabiting crustaceans produce no sounds which would reveal their presence to their prey; the latter would therefore have to rely upon other forms of stimulation for the detection of their foes. Even if it were admitted that they possessed a sense of hearing, yet, as shown both by Beėr's experiments and by my own, it must be so restricted in range that they would be able to detect sound produced at no greater distance than that at which the vibrations could be felt by the hand. Such a dull sense as this would be of no practical value in protecting crustaceans from their foes.

Both observation and experiment lead, then, to the following general conclusions:

(1) The reactions formerly attributed to sound stimuli are nothing more than tactile reflexes.

(2) The otocyst has little or no part in calling forth these reactions.

(3) There is no direct evidence to prove that decapod Crustacea hear, and until such evidence has been obtained, we are not warranted in ascribing to the otocyst a true auditory function.

II. The Otocyst as an Organ of Equilibration.

All water-inhabiting, free-swimming animals which maintain a definite position with reference to gravity either during locomotion or when at rest, can thus orient themselves only under one or the other of two conditions:

Either the animal must be normally in a condition of stable equilibrium, keeping its definite position under the influence of gravity like any inanimate body; or, if a position of unstable equilibrium is maintained, the animal must in some way be made sensible of the direction of gravity, and must keep itself in equilibrium by its own efforts.

In the first case merely the mechanical action of gravity is called into play; in the second instance, besides the outside action of a physical agent, a subjective sense of direction and orientation is involved.

In free-swimming decapods the body, moving or at rest, is in a position of unstable equilibrium. The dorsal side being always kept uppermost, the centre of gravity is high up, and a dead individual or an inanimate object of the same size, form, and disposition of weight would at once turn over. These animals must then by some means be rendered sensible to the direction of gravity, in order to be able to maintain a definite position of unstable equilibrium with reference to it. To determine what are the organs which perform the function of equilibration, the following means have been employed in the present investigation :

(1) Removal, or prevention of the action of an organ, and observation of the effects on the equilibration of swimming or walking decapods.

(2) Observation of the effect of such removal on the gimbol-like movements of the eyestalks (compensation movements) when the animal is rotated about its different axes.

(3) Observations on the orientation of animals normally without otocysts.

(4) The effect of the development of the otocyst on the equilibration of the free-swimming larvæ.

(5) The effect on equilibration of the addition of magnetic attraction acting on the otocyst at right angles to the pull of gravity.

In these experiments blinding was accomplished by painting the eyestalks with a mixture of lampblack and shellac. The otocysts were removed under the lens of a dissecting microscope with the aid of a fine needle, bent in the form of a hook. Other parts, such as flagella of antennæ and antennules, were simply cut off with a pair of fine scissors. Palæmonetes vulgaris, being hardy, was the species chiefly employed, but experiments of a like nature were also carried on with Mysis, Crangon, and Gelasimus. A large number of trials were made with each species. When organs were cut off or destroyed, the animals so operated upon were kept under observation for from 15 to 25 days, and the experiments were then repeated, in order to make sure that the effects observed directly after the operation were not due to abnormal conditions produced by nervous shock.

1. *The Removal of Sense Organs and its Effect on Equilibration.*

The normal position in which a shrimp, like Palæmonetes, holds itself while swimming, is very characteristic:

(*a*) The dorsal side of the body is always kept uppermost, its dorso-ventral axis corresponding to the direction of gravity, and its long axis usually lying in a horizontal plane.

(*b*) Shrimps can be overturned only with difficulty, and even if this is accomplished, they right themselves at once.

(*c*) Animals coming to rest upon surfaces not horizontal tend to keep themselves in the horizontal plane, but with the dorsal side always up.

a. Eyes blinded. Nearly fifty animals were operated upon in this way and their movements observed. Placed in an aquarium, they swam about indiscriminately, but always with the dorsal side up, there being little if any rolling from side to side. They were not easily overturned artificially, and when interfered with, righted themselves quickly. The most noticeable difference to be observed between their movements and those of normal animals was the tendency to remain quiet and to hold fast to any object with which they came into contact, thus substituting the sense of touch for that of vision lost. It is apparent, therefore, that some organ or organs other than the eyes play the chief part in equilibration.

b. Both Otocysts removed. Twenty-five animals were operated upon by removing both otocysts. In swimming there was still a strong tendency to keep the dorsal side uppermost, but there was in every case marked rolling from side to side, which occasionally culminated in a complete rotation about the long axis of the body. The animals could be easily overturned, and though they strove to right themselves, it was not accomplished as soon nor as accurately as in normal or blinded shrimps. They were more apt to remain quiet, or to swim along upon the bottom of the aquarium, than to swim free. If the long flagella of the first and second antennæ were removed, rolling motions were increased and also the difficulty in righting themselves if overturned, the flagella being probably used as balancing organs in equilibration; but the extirpation of the otocysts alone brings about a marked loss of orientation, much more pronounced than that produced by simply blinding.

c. Both Eyes blinded and both Otocysts removed. Upon removal of both otocysts and blinding of both eyes, entire loss of the normal position in swimming resulted in twenty-one trials out of the twenty-five made. The animals turn over and over, rotating about the long axis, now in one direction, now in the other; they also pitch forward and backward about their transverse horizontal axis, and often swim upon their backs. They do not resist overturning, unless holding to some stationary object, and make no attempt to right themselves when swimming free. The moment they come in contact with a horizontal surface, such as the bottom of an aquarium, they at once take up their normal position, righting themselves quickly, but if the surface they touch be oblique or vertical, and even if they come in contact with the under side of a horizontal surface, they cling to it tenaciously, taking up a position *with reference to the plane of contact*, and not in relation to the direction of gravity, as is the case with normal animals. Thus the phenomena of orientation completely disappear in the majority of cases when both otocysts and eyes are rendered functionless, at least in the free-swimming animal. When the animal comes in contact with solid objects, the sense of touch asserts itself and the phenomena of orientation are again, to a certain degree, made manifest.

d. One Eye blinded, both Otocysts removed. The conditions here are essentially the same as when only the otocysts are extirpated. There is a well-defined rolling motion in swimming, and if overturned artificially, the animal is very slow in regaining the original position.

e. Both Eyes blinded, one Otocyst removed. In such experiments no effect was produced different from that brought about by blinding alone. There was no evidence of a tilting of the dorso-ventral axis toward the injured side, as might be expected, if the functions of the two otocysts were co-ordinated. Nor was there during swimming a rotation toward the side from which the otocyst had been removed. We may therefore conclude that in the phenomena of equilibration each otocyst, as well as each eye, acts independently.

As check experiments, both antennules were removed *distal* to the otocysts. No abnormal conditions were produced in swimming movements, the wounds healed, and these individuals lived in aquaria as long as normal animals. Where the otocysts were extirpated, individuals were kept as long as four weeks, and after this interval, when blinded, they gave the same evidences of loss of orientation as they did immediately after the operation.

These observations, made upon Palæmonetes, were found to hold true also for Crangon, Mysis, and lobster larvæ. Experimentation with the fiddler crab gave like results. If blinded and deprived of otocysts, the crabs rolled both forward and backward when walking or running; this effect was still more apparent when the animals were placed in the water.

2. *Removal of Sense Organs and its Effect on the Compensation Movements of the Eyes.*

The following experiments, carried out on Gelasinus pugilator, confirm the work done by Clark ('96). When a crab is tilted to the right or left, forward or backward, the eyestalks tend to keep their original directions, thus seemingly moving through a certain angle. Such movements, which have been observed also for the head and eyes of many vertebrates and insects, are called compensation movements, and the angle of movement, the angle of compensation.

The angle of compensation in the fiddler crab was measured by means of the apparatus described by Clark ('96), a small table to which the animals could be securely fastened and tilted about their chief horizontal axis. A scale ruled to degrees enabled one to read accurately the angle of compensation, and the angle through which the animal was turned. The long eyestalks of the fiddler crab make it easy to determine the angle of the eye movements.

The angle through which the animals were turned was in all cases 45° first to the right, then to the left, about the chief, or longitudinal axis of the body. In each experiment fifty animals were used, the average being taken as the angle of compensation. These animals were most of them kept twenty days, and the angle then measured again, thus guarding against abnormal conditions.

a. Normal Animals. In normal crabs the eyestalks are so held as to make an angle of about 22° with the vertical. The eye movements are always correlated, and if the animal's body is tilted to the right (45°) the right eye makes a compensating movement of 18° upward, the left eye one of 25° upward; rotated to the left, the conditions are just reversed, the right eye now moving through an angle of 25°. The movement of the eye of the side toward which the animal is rotated is in each case less by about 7° than that of the other eyestalk. This is due to interference of the carapace with the eyestalk, preventing its passage through a greater angle.

b. Both Eyes blinded. Tilting either to right or left had the same general effect as in normal animals, but the right eye described an arc of only 13°, the left eye one of 20°, or *vice versa.* There is thus a marked reduction in the angle of compensation, a decrease of about 5°, as compared with normal animals. This shows clearly the extent to which vision enters into the orientation of these animals.

c. Both Otocysts removed. The angle of compensation is here reduced to 3° and 5°, respectively, for the eyestalks on the side toward and from which the rotation takes place. Even without rotation the positions in which the eyes are held are not definite, as they are in animals which possess otocysts. The stalks often make an angle of 40° or more with the vertical, and their movements are no longer correlated. This, together with the marked decrease in the angle of compensation, as compared with that of blinded animals, makes it evident that in equilibration and orientation the otocyst plays a much more important part than does the organ of vision.

d. Both Eyes blinded and both Otocysts removed. On rotation it was found that the compensatory movements of the eyestalks were practically wanting. Two individuals only out of fifty showed movements of from 3° to 5°. In the greater number of cases no movement could be detected, and in the remainder the angle averaged less than 1°. There was a still greater tendency for the eyestalks to be held in indefinite positions when at rest, and at unequal angles. Fifteen such individuals were kept in an aquarium more than twenty days, and after this lapse of time practically the same results were obtained, showing that the shock of the operation of removing the otocyst had no effect upon the results of the experiments. Furthermore, removal of the antennules distal to the otocysts had absolutely no inhibiting effect upon the movements of the eyestalks.

This series of experiments corroborates, as far as they go, the conclusions of Clark ('96). It is clear from them that the otocyst is the chief organ in equilibration, though sight also plays an important part in the orientation of these animals.

Since the above work was done (July, 1899) a paper has been published by Lyon ('99) on the comparative physiology of compensatory movements. These movements were studied by him in many vertebrate and invertebrate forms; they were found to exist in insects as well as Crustacea. Using the crayfish, he confirms Clark's results to some extent, but finds that on blinding the animals and removing the otocysts a considerable angle of compensation still persists. This, together with the fact that insects, which lack otocysts, show the characteristic movements, he uses as an argument against the otocyst being an organ of equilibration. Lyon also finds that upon rotation about a vertical axis there is a compensation movement of the eyestalks of the normal crayfish through an angle of 10° to 18°; and, further, that when the animal is rotated about its long axis blinding causes a diminution of 10% in the angle of compensation. His results therefore give a much more important place to vision in orientation than do the conclusions of Clark and myself. However, from the combined results of the experiments of Clark, Lyon, and myself, one cannot avoid the conclusion that, in the fiddler crab at least, the otocyst is by far the most important organ in equilibration; next in order comes vision, and then muscular and tactile sense.

3. *Equilibration of Animals normally without Otocysts.*

Virbius zostericola, a shrimp quite common at Wood's Hole, Mass., does not possess otocysts. Observation and experiment brought out several interesting facts concerning it. In the first place, it is not a free-swimming form. Its normal habitat is on the eel grass, to which it clings in positions indifferent to the direction of gravity. When forced to swim, it does so in a very uncertain manner, with the dorsal side usually uppermost, though this is a position of unstable equilibrium. If overturned artificially (and this is easily accomplished), it rights itself slowly and will cling to the first object it may chance to touch. Removed from its supporting blades of eel-grass, its unstable manner of swimming closely resembles that of shrimps in which the otocysts have been destroyed. If the eyestalks are painted with lampblack, and the animals so treated are placed in a large aquarium, and forced to swim, apparently all sense of direction and means of orientation are lost.

4. *The Effect of the Development of the Otocyst on the Equilibration of Lobster Larvæ.*

As has been shown in the morphological part of this paper, there is no otocyst in the newly hatched larva of either Palæmonetes, the lobster, or the crab, nor is there a functional organ during the first three larval stages. It begins to invaginate only in the second larval stage, and it is merely a shallow cup-like depression in the third stage; not until the next moult do the sensory hairs and otoliths appear.

When we examine the conditions as to equilibration and manner of swimming in the different larval stages, we find that in the first larva the body is not definitely oriented while swimming. Newly hatched lobsters are very unstable in their movements, often swim or come to rest upon their backs or sides, and show a tendency to roll from side to side while swimming. The animal swims by means of the exopods of the thoracic appendages; the abdominal segments are flexed ventrally, and the thoracic endopods, hanging down, steady the rolling motions somewhat. In the second stage the conditions are essentially the same.

In the third stage the larvæ are more stable, though the otocyst is still functionless. This greater stability is explained when the swimming position of the body and appendages is observed (Fig. *B*). The thoracic appendages are now relatively large, as compared with the size of the body. They are allowed to hang down ventrally, and in conjunction with the curved condition of the abdominal segments, serve to lower the position of the centre of gravity in the whole animal, thus rendering its swimming position much more stable.

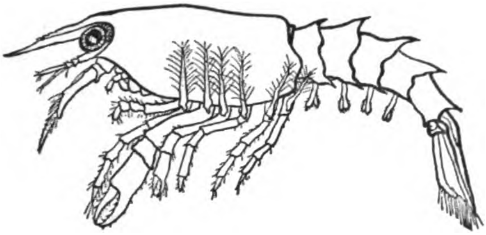

FIGURE *B*.

Lateral view of lobster larva of the third stage, showing swimming position. Magnified 6 diameters.

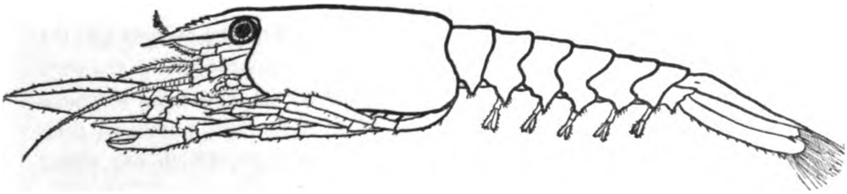

FIGURE *C*.

Lateral view of lobster larva of fourth stage, illustrating the change in swimming position due to the presence of a functional otocyst. Magnified 6 diameters.

Turning now to the fourth larval stage, we find the swimming position of the body entirely changed (Fig. *C*). The abdomen is no longer flexed and curved ventrally, but is held in approximately the same horizontal plane as the cephalothorax, while the thoracic appendages, instead of dragging downward through the water, are held up and forward in a line parallel with the long axis of the body. The great chelæ project in front like the arms of a person preparing to dive, the exopods of the thoracic appendages have been lost, and the larvæ now swim swiftly by means of the abdominal swimmerets.

Although, from the position in which the body and appendages are held, the larva is in unstable equilibrium, it now orients itself very definitely during locomotion, in sharp contrast to the preceding stages. All signs of rolling from side to side, or pitching forwards, are completely lost. The larva swim straight ahead with the body held usually in a horizontal plane and dorsal side up. The same position is also invariably maintained when the animals come to rest.

Thus this sudden change as to form and swimming position in the fourth larva, unfavorable though it is for equilibration, is yet accompanied by more delicate powers of orientation, and greater stability in swimming than are met with in the three earlier stages, where the centre of gravity of the animal is lower. Bearing in mind the fact that the otocyst first becomes functional in the fourth larval stage, we can only conclude that an intimate connection exists between its appearance as an active organ, and the delicate static sense which is suddenly exhibited by the larvæ.

If larvæ of the first, second, and third stages are blinded, their powers of orientation are almost entirely lost, but the same experiment has little or no effect upon the equilibration of the fourth larva. The first three stages thus depend mainly on vision for their imperfect orientation; in the next stage this function has been largely transferred to the otocyst.

A similar correlation between the development of the otocyst and the appearance of a static sense is found in the metamorphosis of the crab. The pelagic unstable Zoea larva is without otocysts, while the Megalops larva, which exhibits perfect powers of equilibration, possesses these organs well developed, and even containing otoliths, which are absent in the sac of the adult.

The correlation which evidently exists between the formation of the functional otocyst and the sudden increase in static powers exhibited by lobster larvæ is particularly well shown in the marked alteration in the swimming position maintained by the fourth larva, as compared with that of the three earlier stages. Previous to the fourth stage, the lack of a delicate static organ is compensated for by the maintenance of an attitude in swimming which increases the stability of the moving body. Just as Bethe ('95) found that Mysis, deprived of its otocysts, would after an interval of some days recover its power of orientation by curving the abdomen upward and thus, by lowering the centre of gravity, put the body in natural equilibrium, so in the case of the first three lobster larvæ, the attitude maintained is an adaptation for the greater stability of the free-swimming animal, as yet without static organs. But when, in the next stage, the otocyst becomes functional, such an adaptation is no longer necessary, and the sudden change to the unstable swimming position of the fourth larval stage results (Fig. *C*). This is the more natural attitude, and is advantageous to the animal in that it allows of greater speed in swimming.

5. *The Function of the Otoliths.*

At the time when the otocyst was regarded as an auditory organ, the otoliths were supposed to act simply as intensifiers of the sound vibrations, but viewing the sac as a static organ, the rôle played by the otoliths must assume a different aspect. The fact that they are wanting in the Brachyura, which nevertheless exhibit strong powers of orientation, might be used as an argument against their playing any important part in equilibration. But as they are present in the larval crab, and as they disappear only when the otocyst becomes highly differentiated, and when sensory hairs much more delicately constructed than the otolith bristles are developed, this argument loses most of its weight.

For determining the functions of the otoliths two methods may be employed : (*a*) Observing the effect on equilibration and orientation following the removal of the otoliths, or the prevention of the normal process of taking them in after ecdysis. (*b*) Substitution of iron dust or iron filings for the otoliths, and the employment of an electro-magnet to modify the action of gravity. If the otoliths are static in function, the animals should orient themselves with reference to the resultant of the attraction of the magnet, and the pull of gravity.

The first of these methods was attempted by Kreidl ('94), but failed, as he was unable completely to remove the otoliths. His results with the second method of experimentation were definite and affirmative. Lyon ('99) attempted to repeat and verify Kreidl's work, but his experiments were incomplete and negative in their results.

Otoliths, always normally present in macruran decapods, are lacking for only a short time after ecdysis. So short indeed is this interval, that it is extremely difficult to find otocysts of newly moulted animals which are without otoliths. Nor is it usually possible to prevent a crustacean which has been observed to cast its test, from getting new otoliths into the sac ; at least not for a sufficiently long period to allow the animal otherwise to regain its normal condition. Even if placed at once in filtered water, some otoliths soon make their appearance, probably originating from the excreta of the animals themselves.

In lobsters the larvæ regain their normal condition within a much shorter interval after ecdysis than do adult individuals; their digestive tract is also much less likely to contain material suitable for the formation of otoliths. Therefore, after trying in vain to completely remove the otoliths from the sacs of Crangon and Palæmonetes, my attention was directed to lobster larvæ as much more favorable material than the adult shrimps. As they moult at intervals of a few days, it is also much easier to obtain them directly after ecdysis or in the very act itself. So obtained, and placed at once into filtered sea water, larvæ of the fourth stage may be kept without otoliths for from twenty-four to forty-eight hours, and a favorable opportunity is thus given for observing the effect produced by the lack of otoliths on the equilibrium of the animals.

Observations were made on eighteen larvæ of the fourth and fifth stages, all of them being kept free from otoliths for at least twelve hours. Within two hours after moulting most of them swam about actively, and ate greedily when fed with bits of crab's liver. In swimming, however, they show distinctly the phenomena manifested by shrimps which have been deprived of their otocysts. There is both "rolling" from side to side, and "pitching" forward and backward; often they swim with the ventral side uppermost. Much more easily overturned than normal larvæ, they do not right themselves at once, but if turned upon the back, will continue to swim in that abnormal position. If blinded, the loss of equilibrium is still more marked. All these conditions are in strong contrast to the actions of the normal free-swimming larvæ of these stages, which conduct themselves in the characteristic manner already described for Palæmonetes.

The observations having been made and recorded, the animals were killed, and the otocysts dissected out and examined under the microscope. Scarcely a particle of inorganic matter was found in the sacs of sixteen larvæ. In two individuals a few small grains of sand were found in one otocyst, but the other was entirely destitute of otoliths.

From the number of cases observed it seems safe to conclude that the otoliths do play an important part in equilibration, and that it is the pull of gravity upon them which stimulates the sensory hairs of the sac. If the loss of the power of accurate orientation were to be attributed to the abnormal conditions resultant upon ecdysis, it might be said in reply that the larvæ were perfectly normal when observed, as far as feeding and active swimming were concerned, and furthermore that the loss of equilibration disappeared at once when a larva without otoliths was allowed to obtain them. The results of these observations are also confirmed by the following experiments.

The otoliths were removed from the sacs of Palæmonetes by lifting the lid which covers the aperture, and forcing a fine jet of water into the cavity. Most of the sand having been thus washed out, the animals were placed in an aquarium upon the floor of which iron filings had been scattered and were allowed to remain until the iron particles had been taken into the sac in place of grains of sand. As an electromagnet, a steel bar 8 inches long and one quarter of an inch square was used. This was ground down nearly to a point at one end; about the other end were wound many layers of fine copper wire, the termini being connected with the circuit of a small six-celled battery. The shrimps employed in the experiments (Palæmonetes) were blinded by the usual method, — painting the eyestalks with a mixture of lampblack and shellac. The pointed end of the magnet was held about 3 cm. from the otocysts, at one side of and a little ventral to them. Animals with normal otoliths, if blinded, do not respond at all, and are apparently unaffected by the proximity of the magnet; they keep their normal position, dorsal side up, with the sagittal plane of the body coincident with the direction of gravity. If not blinded, they simply move slowly away from the magnet when it approaches too near. When, however, the magnet is brought into close proximity to otocysts containing iron filings, the dorsal side of the animal is turned, not toward the magnet, as might be expected if the changed position were due directly to the action of the magnet on the iron filings, but away from it. If the magnet was changed to a position on the other side of the shrimp, the turning was in the opposite direction, still away from the source of attraction.

The above reaction was distinctly noted a number of times for each of the six animals experimented upon. As Kreidl's work was fairly complete, only one series of experiments was tried in confirmation of his results. When the observations had been completed, the antennules of the six shrimps were removed and the otocysts examined under the microscope. In each case particles of iron were found nearly filling the sac, and if a magnet was held close to one of the latter, the whole antennule was lifted by the attractive force, showing clearly that there must have been an effective magnetic pull upon the otoliths of the live animals during the experiments. I believe there is only one explanation for this turning of the body away from the attracting force, and that is a very simple one. Under normal conditions the body of the shrimp is oriented with reference to gravity, and its dorso-ventral axis approximately corresponds to the direction of this force. If the shrimp rotates around its chief axis either to right or left, say 90°, the direction of the pull of gravity on the otoliths is at once changed, and through the medium of the latter other sensory hairs of the sac are stimulated. As a result, the shrimp turns back in a direction opposite to that in which it was rotated, until it is again in a normal relation to the direction of gravity. The employment of the magnet has no other effect than merely to change the direction of the orienting force. This is now no longer that of gravity alone, but *the resultant* of the two component forces, gravity and the pull of the magnet. The animal now maintains its swimming position in reference to this new line of attraction, its dorso-ventral axis coincident with that line, and as a result the dorsal side is turned away from the magnet. To put it in another way, when the magnet is held close to the right side of the otocyst, the animal is stimulated precisely as it would be if rotated to the right 45°, and it responds as it would normally in righting itself, i. e., by turning its body in the opposite direction through an angle sufficient to make its dorso-ventral axis coincide with the direction of the attractive force; in this case through an angle of 45°.

This single series of observations completely confirm, as far as they go, the very important conclusions of Kreidl. The otoliths are found to play an important part in the functional activities of the otocyst, and the latter is conclusively proved to be a static organ, acted upon by the force of gravity; this force makes itself felt chiefly through the medium of the otoliths, and if they are absent, as described in a preceding set of experiments on lobster larvæ, the function of the otocyst in Macrura is seriously impaired.

6. *The Function of the Hairs of the Otocyst.*

The function of the otocyst hairs of macruran decapods which are in contact with otoliths has been already briefly discussed in the first part of this paper. The stimulus imparted to the hair shaft through the medium of the otoliths makes itself most strongly felt at the labile base of the hair, owing to the rigidity of the shaft and the delicacy of the attaching membrane. At this point, too, the nerve fibre invariably ends, and the stimulus is thus transmitted to it, and at once carried to the brain. In the case of adult Brachyura, however, there are no otoliths in contact with the hairs of the otocyst, consequently the effect of gravity, if not entirely null, must be at least greatly lessened, unless indeed the hairs are so differentiated as to be themselves stimulated by it.

Bethe ('97), acting on the idea that in tilting the animal the difference in the pressure of the water might affect the hairs of the otocysts, placed crabs under very high pressures where the slight difference brought about by tilting would be practically eliminated. But he found that all the phenomena of equilibration still persisted.

It is probable that in the otocyst of Carcinus the thread hairs are the most important sensory organs of the sac. The hook hairs, originally in the larva attached to otoliths, later, with the loss of the sand granules, lose much of their functional activity; the third group of hairs cannot be of great importance, as I could not demonstrate satisfactorily their nerve connections, and their structure alone is such as to preclude their being affected by very delicate stimuli. The thread hairs, however, in both structure and position are fitted for the fulfillment of such a function as has been ascribed to them. The shaft is long, attenuate, only slightly fringed at the tip, and attached at the base by a very thin membrane, which allows free movement to the rigid shaft about this region as upon a joint. I have observed in studying freshly dissected otocysts that a slight tilting of the watch glass in which they were contained caused these hairs to sway extensively.

From Clark's experiments and my own, it was apparent that upon rotation in a horizontal plane, there was little or no compensatory movement of the eyestalks, and that when there is such a reaction, the angle of compensation is not maintained, but the eyes return at once to their original positions. Also, on rotation about the animal's lateral axis, the angle of compensation is not as great, when the rotation is rapid and jerky, as when performed slowly and smoothly. These two facts preclude the possibility of the hairs being affected by movements of the fluid surrounding them, at least to any great extent. For if they were so affected, the angle of compensation should be the same, in whatever plane the animals are rotated, and the position of the eyestalks should be in every case maintained by compensation movements.

There still remain two ways in which the hairs may be so affected as to bring about nervous stimulus. Either they may be lighter than the surrounding fluid, and consequently tend always to float erect, no matter what position the otocyst may take relative to them; or they may be heavier than the liquid contents of the otocyst, in which case they would be affected by gravity directly, and exposed to a greater or less pull according to their different positions in the sac. My observations made on dissections of fresh material of both young and adult crabs, do not confirm the first of these hypotheses. The hairs rarely, if ever, float upright in the fluid of the otocyst; on the contrary they usually project out horizontally, with their tips a little lower than their bases; and such conditions would favor the second supposition, that they are heavier than the surrounding fluid. Unfortunately, when fresh material was at hand, my attention was directed toward other problems, and no dissections or observations were made with the settlement of this question primarily in view. It is, however, a point well worth future experimentation, for the function of these hairs is apparently similar to that of the auditory hairs of the vertebrate cristæ acusticæ, and to clearly show how they are stimulated would throw light on an important problem in the physiology of the vertebrate ear.

SUMMARY.

1. The cuticular lining of the otocyst, found in the basal segment of the antennule of all decapod Crustacea, is cast with the test at each moult. It is composed of thin chitin, and is suspended from the dorsal wall of the antennule, which presents an aperture in Macrura, in the larval stages of Brachyura, and also in adult Brachyura directly after ecdysis.

2. In Macrura a single sensory prominence is present, either on the floor or sides of the sac. In Brachyura there are three sensory regions. The sensory hairs are borne upon these cushions, usually in curved rows.

3. The otolith hairs are heavily fringed, often bent or hooked. In Macrura they are attached to the wall of the sac by a thin bulb of chitin; in Brachyura the base of the hair shaft is inserted into a cup-like depression; both methods of attachment allow the hair to sway freely upon its base.

4. The free hairs of the otocyst, found in the lobster and all Brachyura, are extremely long and attenuate; their basal attachment is delicate, and renders them much more sensitive than the otolith hairs.

5. All sensory hairs are formed as double-walled tubes by numerous matrix cells situated beneath the hypodermis, from which they originate. After ecdysis processes from these cells extend into the shaft of the newly formed hair. In preparation for the next moult these processes

are withdrawn, the matrix cells recede from the base of the old hair, and arrange themselves about the nerve fibre for the formation of the new bristle. There is a period between moults, more or less extended, during which no living substance is present in the greater part of the cavity of the hair.

6. The otoliths are grains of sand taken in from the exterior (first, in the case of the lobster, by the fourth larva) and renewed after each moult; they may lie free in the otocyst, or be attached to the sensory hairs. In Brachyura they are found only in the *Megalops* stage.

7. Glands similar in structure to the tegumentary glands are present in the lobster and crayfish beneath the sensory cushions which bear otolith hairs. They secrete a substance for the attachment of the otoliths to the pinnules of the bristles.

8. The innervation of the otocyst hairs and olfactory bristles is distinctly unlike.

(*a*) The otocyst hairs have each a single nerve element, and the terminal fibre ends in the enlarged base of the shaft without branching.

(*b*) Each olfactory bristle is innervated by numerous ganglion cells (100 or more). The peripheral strand of fibres from these cells extends some distance into the cavity of the hair, terminating free and without modification of any kind.

9. The central terminations of all the otocyst fibres are in two closely connected neuropilar masses at the posterior end of the brain, median to those of the second antennæ, and ventral to the optic centres. The nerve sheaths disappear as the fibres enter the "Punktsubstanz," and the fibrillæ soon separate. They cannot be traced to determinate endings, nor are they ever directly connected centrally, with ganglion cells.

10. Each sensory nerve fibre is composed of numerous fibrillæ, embedded in a semi-fluid "perifibrillar" substance, which in turn is surrounded by a delicate sheath. The flowing together of the perifibrillar matrix causes the beaded or varicose appearances characteristic of methylen blue, and silver impregnations. The fibrillar structure can be demonstrated in both the central and peripheral portions of the fibres.

11. The sensory ganglion cells are all typically bipolar and elongate in form. They are placed at some distance from the base of the hair which they supply, and show no fibrillar structure.

12. In the shrimp-like decapods, such as Palæmonetes and Crangon, a nucleated myelin sheath surrounds each sensory fibre and ganglion

cell, extending from the neuropil of the brain nearly to the peripheral ending of the fibre.

13. Each sensory ganglion cell with its central and peripheral fibres constitutes a single nervous element or neuron. The neurons are trophic units, and direct connection between two neurons was not demonstrated.

14. In those decapods which pass through free-swimming larval stages, the otocyst develops as an invagination of the dorsal ectoderm of the basal segment of the antennule, and becomes functional only at the fourth moult after hatching.

15. Invagination begins at the second larval stage, but the matrix cells which are to form the sensory hairs of the sac, make their appearance in the first larva, being derived from the cells of the hypodermis.

16. During the third stage the sensory hairs are formed below the floor of the shallow sac; at the next moult these become functional, the sac enlarges, and otoliths make their appearance. The otocyst is now functional, the hairs are innervated as in the adult, and more than 100 of them may be present. After the fourth stage the chief changes are the increase in the number of otocyst hairs, and the gradual constriction of the orifice of the sac.

17. In Brachyura the Zoea larva is without a functional sac. In the Megalops the otocyst is open, and contains numerous sensory hairs and otoliths. During the next two stages the aperture closes and takes on the adult condition, without otoliths.

18. Structurally, the otocyst of decapods may be compared roughly to the utriculus of such a vertebrate as Myxine; the sac of Palæmonetes to a single isolated ampulla, and its sensory cushion to a crista acustica. The closed otocyst of Brachyura has three sensory regions and is without otoliths. It therefore approaches in general structure the utriculus of the higher vertebrates. Each sensory element of the otocyst is comparable to a single sensory component in the vertebrate crista. In each there is a modified organ for the reception of stimuli, connected basally with the terminal fibre of a sensory neuron.

19. There is no part of the decapod otocyst which is structurally comparable to the middle ear, semi-circular canals, or cochlea of vertebrates.

20. There is no direct evidence to prove that decapod Crustacea react to true sounds produced either in water or in air. The reactions formerly attributed to audition are probably due to tactile reflexes.

21. The otocyst plays little or no part in calling forth these reactions, and does not function as a true auditory organ.

22. Equilibration is made possible by three sets of organs, the otocysts, the eyes, and the tactile bristles.

23. In free-swimming decapods the otocyst is by far the most important of these static organs functionally, vision being secondary to it. Four facts go to prove this:

(*a*) The removal of the otocysts causes a much greater loss of power of orientation, and a greater decrease in the compensatory movements of the eyestalks, than the loss of vision.

(*b*) Decapods and Entomostraca normally without otocysts either swim in unstable equilibrium, or in a position identical to that which an inanimate object of the same form and weight would take under the influence of gravity.

(*c*) Lobster larvæ without functional otocysts are unstable in their swimming movements, but orient themselves with great accuracy at the stage when the sac becomes an active sense organ.

(*d*) If iron filings are substituted for the otoliths, and an electromagnet is employed to modify the effect of the pull and direction of gravity, shrimps orient themselves with reference to the direction of the resultant pull of the two forces, precisely as they do to the attraction of gravity alone.

24. In lobster larvæ of the third and fourth stages there is a direct correlation between the metamorphosis of the otocyst from a functionless to an active organ, and the changes in the swimming position of the animal. When the sac is inactive (third stage), the swimming position of the body and appendages is an adaptation which places the larvæ in comparatively stable equilibrium. As the otocyst becomes functional (fourth stage), this adaptation is no longer necessary, and a much less stable position is maintained, but one more favorable for rapid locomotion.

25. The otoliths in Macrura and larval Brachyura are the means by which the pull of gravity is transmitted to the hairs of the otocyst. On their complete removal there is loss of equilibration and power of orientation; if iron filings are substituted for them, shrimps may be made to respond to the attraction of an electromagnet.

26. In adult Brachyura otoliths are normally lacking. The otolith hairs have become practically functionless, and the thread hairs are modified in such a way as to make them directly responsive to the attraction of gravity without the aid of the otoliths.

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EXPLANATION OF THE PLATES.

All Figures were outlined with an Abbé camera lucida. Tube length was usually 160 mm., with projection distance to the table, 410 mm. The magnifications are given with the descriptions of the several figures. Drawings were made from sections, unless the contrary is stated. The orientation of the figures is given for each plate.

ABBREVIATIONS.

cl. gl.	Gland cell.
cl. gn.	Ganglion cell.
cl. ma.	Matrix cell.
co'nt. crc'oes.	Circumœsophageal connective.
crs. sns.	Sensory crista.
cta.	Cuticula.
cta'.	New cuticula.
dt.	Duct.
fbr'.	Fibrillations.
fbr. ass.	Association nerve fibre.
fbr. c.	Central nerve fibre.
fbr. n.	Nerve fibre.
fbr. pi'ph.	Peripheral nerve fibre.
fg.	Flagellum.
glb.	Globulus.
gn. olf.	Olfactory ganglion.
h'drm.	Hypodermis.
lab. a.	Anterior lip of orifice to otocyst.
lab. p.	Posterior lip of same.
lu.	Lumen of otocyst.
mal.	Hammer.
mb. sph.	Spherical membrane.
n. at.1	Antennular nerve.
n. at.2	Nerve of 2d antenna.
n'bl.	Neuroblasts.
n'lem	Neurilemma.
nl. tu.	Sheath nucleus.
n. opt.	Optic nerve.
n. ot.	Otic nerve.
n'pil. at.1	Neuropil of antennule.
n'pil. at.2	Neuropil of antenna.
n'pil. opt.	Optic neuropil.
n. teg.	Tegumentary nerve.
of.	Orifice.
ot'cy.	Otocyst.
ot'lth.	Otoliths.
pinn.	Pinnules of hairs.
pr'c. pr'pl.	Protoplasmic process.
rm. l.	Lateral branch of antennular nerve.
ro.	Rostrum.
set.'	Group hairs.
set. fil.	Thread hairs.
set. l.	Lateral hairs.
set. m.	Median hairs.
set. olf.	Olfactory hairs.
set. ot.	Otic hairs.
set. p.	Posterior hairs.
set. ta.	Hook hairs.
set. tac.	Tactile hairs.
tb. set.	Hair tube.
tct.	Tectum of otocyst.
tu. myl.	Myelin sheath.

PLATE 1.

All Figures are of *Palæmonetes*. In Figure 1 anterior is up on the plate; in Figures 2, 3, and 4 the dorsal side is up, and the anterior end in Figure 4 is at the left. Lines numbered *2*, *3*, *4*, *5* in Figure 1 indicate the planes of the sections shown in Figures 2, 3, 4, and 5 respectively.

Fig. 1. Dorsal view of the basal segment of both antennules, showing otocysts and the arrangement of the hairs in the sac. × 30.

Fig. 2. Somewhat oblique transverse section, extending from dorsal anterior to ventral posterior, of both antennules and the rostrum, through the posterior ends of the otocysts (compare line *2*, Fig. 1). × 64.

Fig. 3. Transverse section of right antennule through the orifice of the sac, showing tectum and otoliths (compare line *3*, Fig. 1). × 64.

Fig. 4. Parasagittal section through right antennule and brain, showing the course of the otic nerve, with a single nerve element drawn diagrammatically (see line *4*, Fig. 1). × 64.

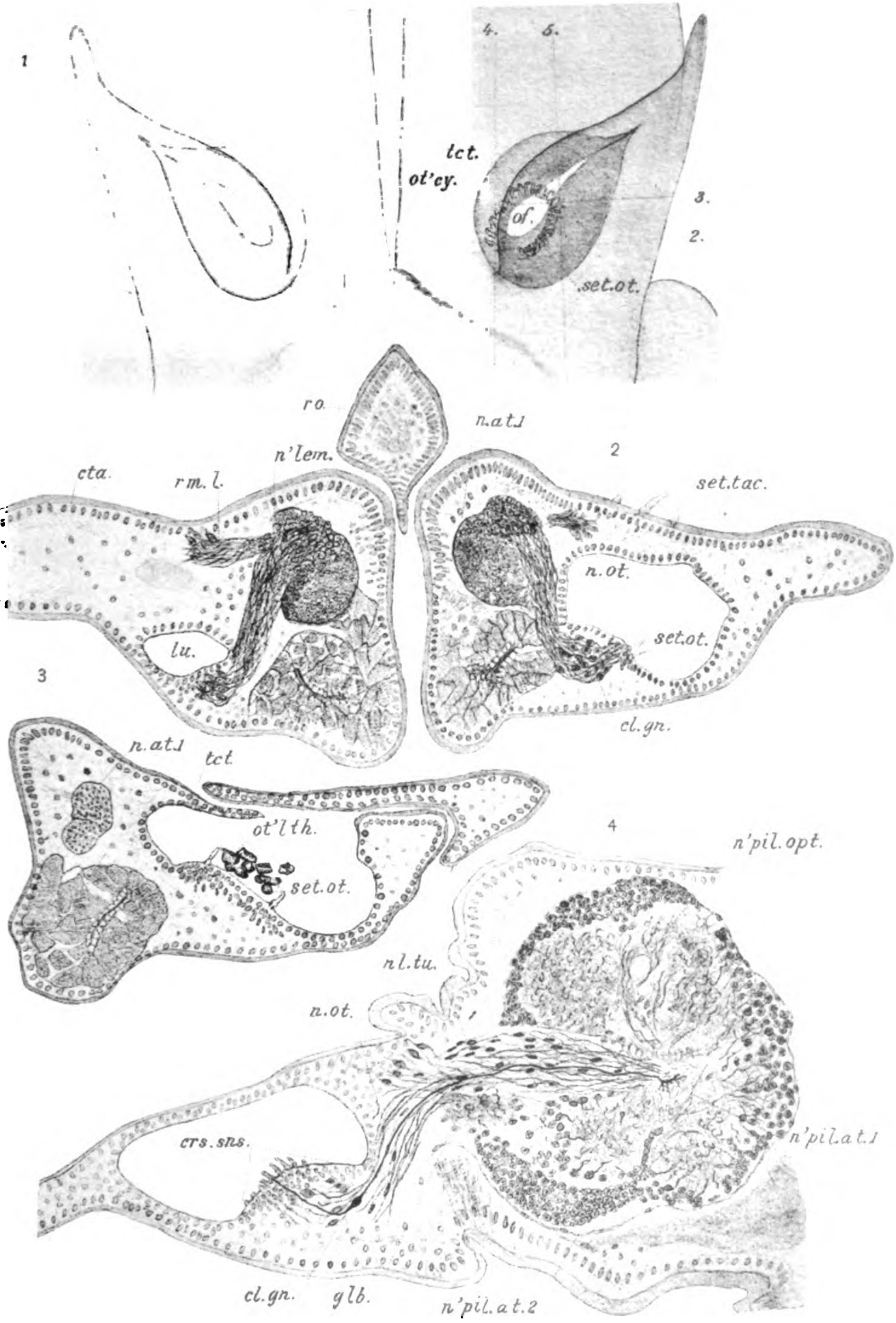
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PLATE 2.

All Figures are of *Palæmonetes*. The dorsal side is up in Figure 5, and the anterior end at the right.

Fig. 5. Parasagittal section through the lateral side of the right otocyst (see line *5*, Fig. 1). × 64.

Fig. 6. Transverse section through the posterior end of the sensory cushion, showing two lateral hairs, the base of a median one and a group of ganglion cells. × 168

Fig. 7. Otocyst hair and matrix cells. × 600.

Fig. 7a. Another otocyst hair and matrix cells. × 600.

Fig. 8. Sensory hair of the otocyst and the ending of its peripheral nerve fibre. × 600.

Fig. 9. Fundament of developing sensory hair from an abdominal exopod, showing matrix cells about the nerve fibre. Methylen-blue preparation. × 600.

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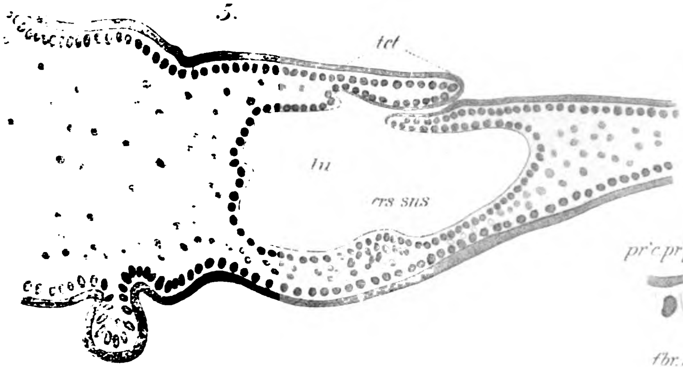
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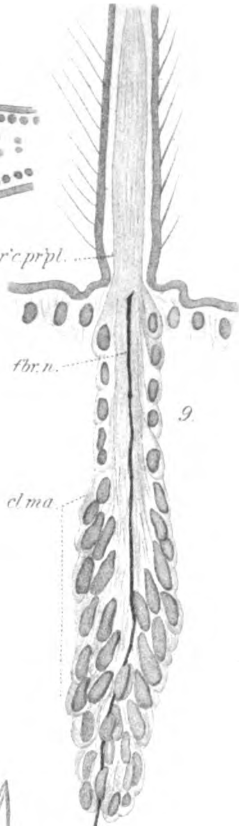
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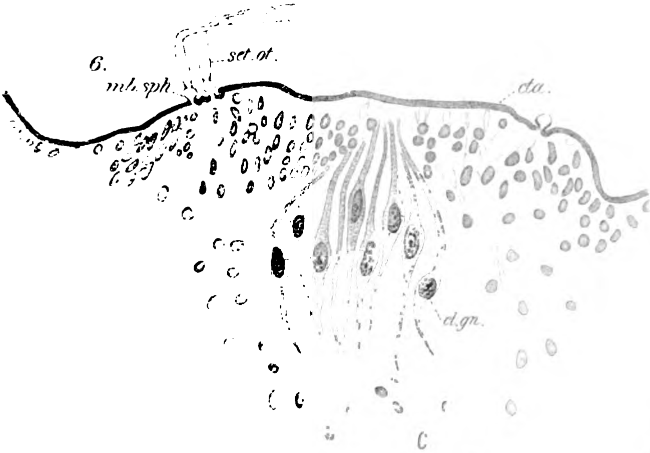
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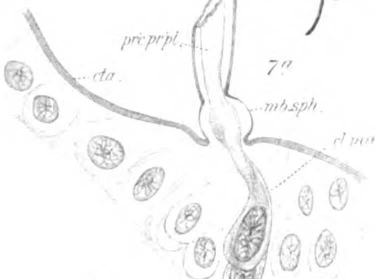
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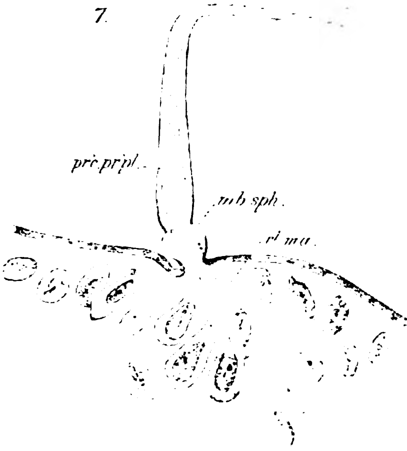
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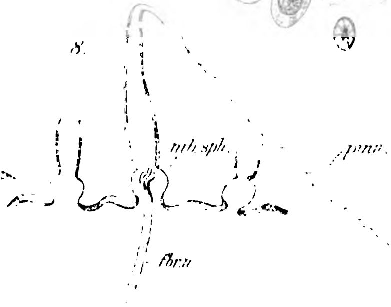
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PLATE 3.

All Figures are from methylen-blue preparations of *Palæmonetes*. Anterior is up on plate in Figure 12.

Fig. 10. Part of abdominal exopod showing tubes of developing tactile hairs and their innervation. × 125.

Fig. 11. Peripheral nerve endings in the tactile hairs of the second maxilliped. × 95.

Fig. 12. Dorsal view of antennules and brain, showing sensory neurons and central endings of the otocyst nerve fibres. The peripheral endings in the left otocyst are diagrammatic. × 30.

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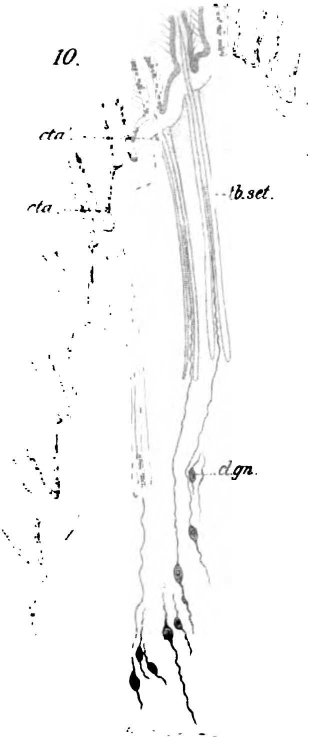
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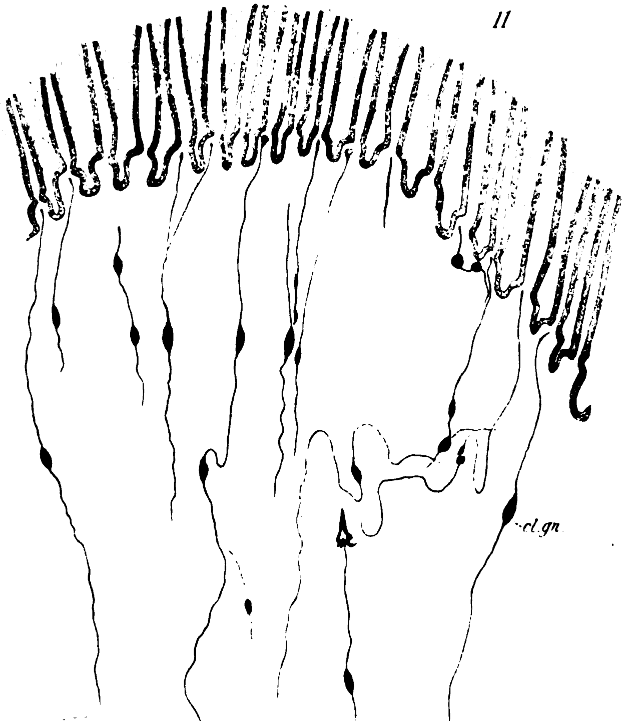
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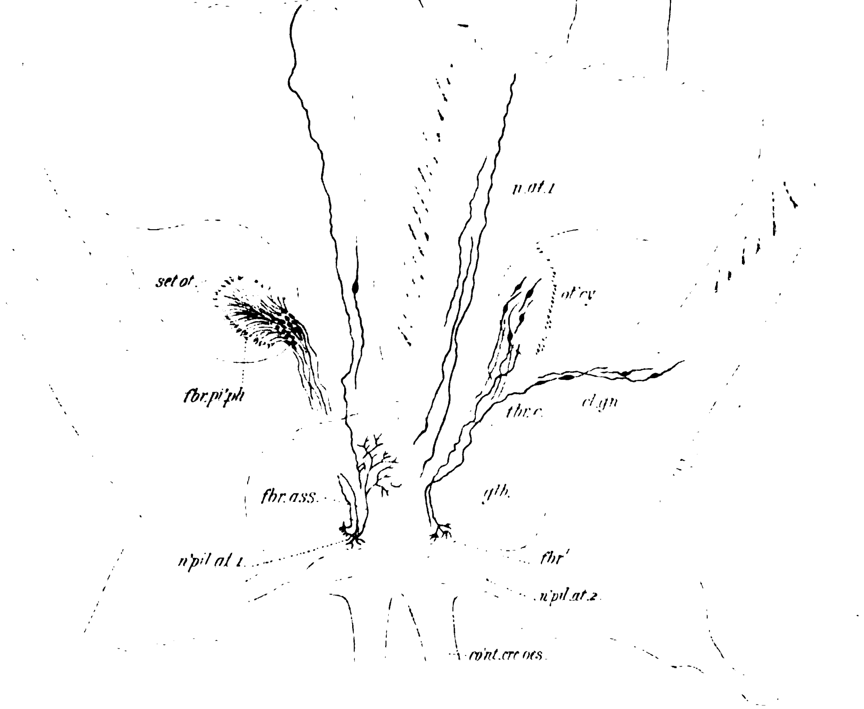
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PLATE 4.

Figures 13–18 are of *Palæmonetes.* Figures 19–22 are of *lobster larvæ.* In Figures 19–21 dorsal side is up and the lateral side is at the right; in Figure 22 anterior is at the right, dorsal side up.

Fig. 13. Portion of inner flagellum of first antenna, showing olfactory hairs and their peripheral ganglionic masses. Methylen blue. × 125.

Fig. 14. Gustatory hairs and nerve elements from the basipod of second maxilla. Methylen blue. × 95.

Fig. 15. Fibrillations in peripheral otic nerve fibre. Methylen blue. × 1300.

Fig. 16. Ganglion cell of otocyst and peripheral nerve process surrounded by myelin sheath. × 600.

Fig. 17. Ganglion cell of otocyst, and sheath nucleus. × 1300.

Fig. 18. Ganglion cell of otocyst. Methylen blue. × 770.

Fig. 19. Transverse section through right antennule of first lobster larva. × 168.

Fig. 20. Transverse section through right antennule of second lobster larva; beginning of invagination. × 168.

Fig. 21. Transverse section through right antennule of third lobster larva. × 168.

Fig. 22. Parasagittal section through antennule of second lobster larva. × 168.

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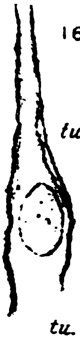

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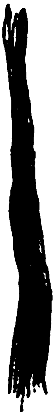

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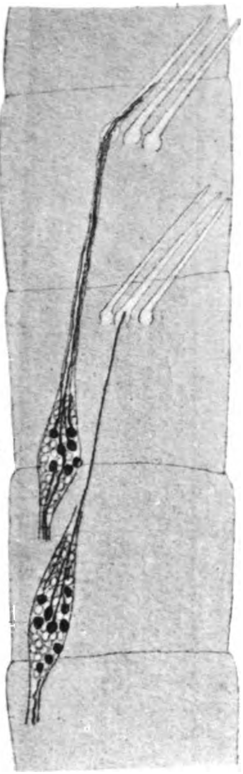

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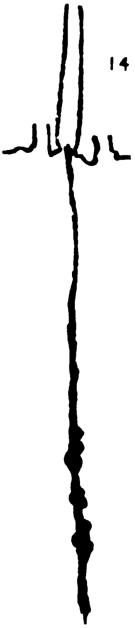

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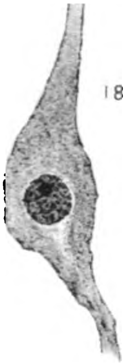

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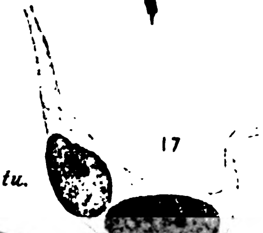

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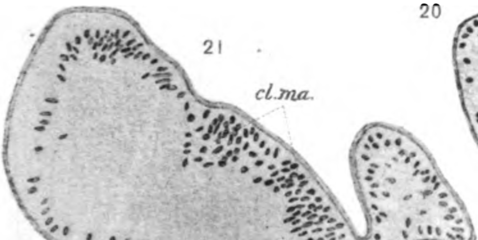

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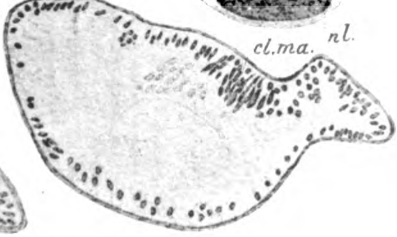

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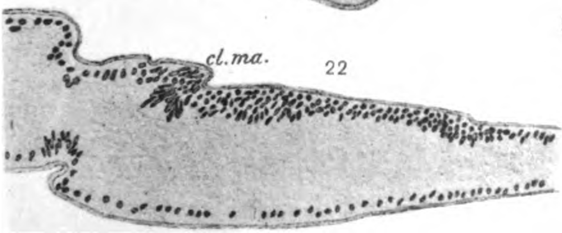

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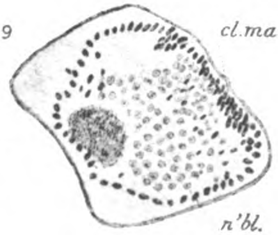


PLATE 5.

All Figures are of *lobster larvæ*. In Figures 23 and 25 dorsal is up, and anterior at the right; in Figure 24 dorsal is up and lateral is at the right; anterior is at the right in Figure 26.

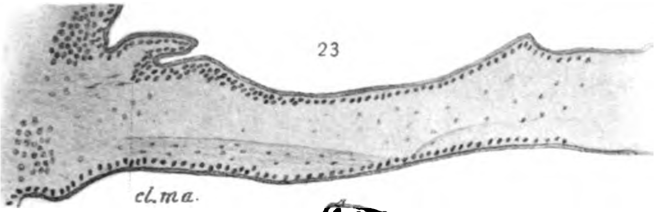
Fig. 23. Parasagittal section through the antennule of the third larva. × 168.

Fig. 24. Transverse section through the posterior part of the right otocyst in fourth larva. × 168.

Fig. 25. Parasagittal section of the same. × 125.

Fig. 26. Diagrammatic dorsal view of floor of right sac dissected out to show arrangement of the hairs and their innervation. Methylen blue. × 168.

Fig. 27. Developing hairs of the otocyst in the third larval stage. × 600.



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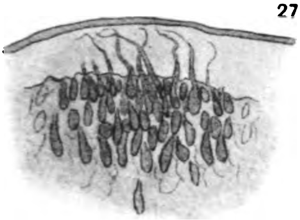

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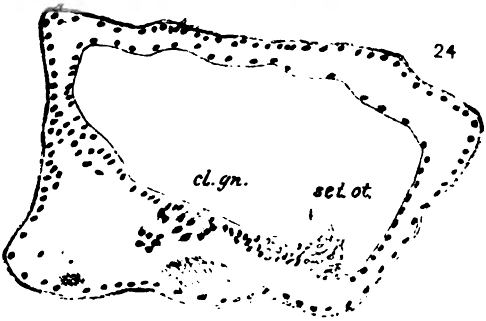

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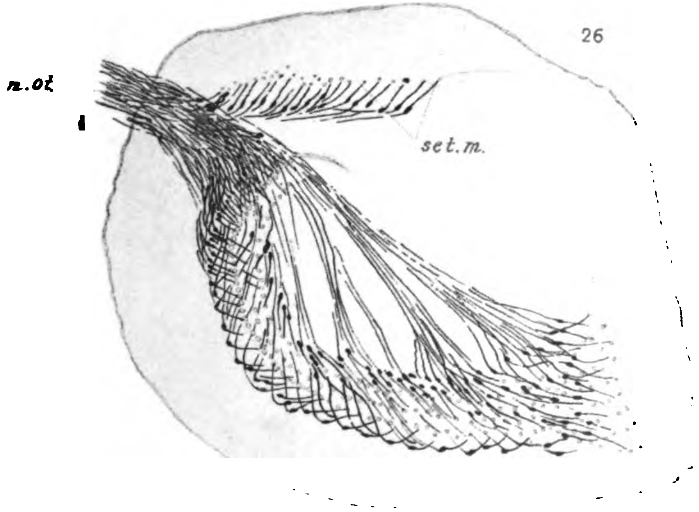

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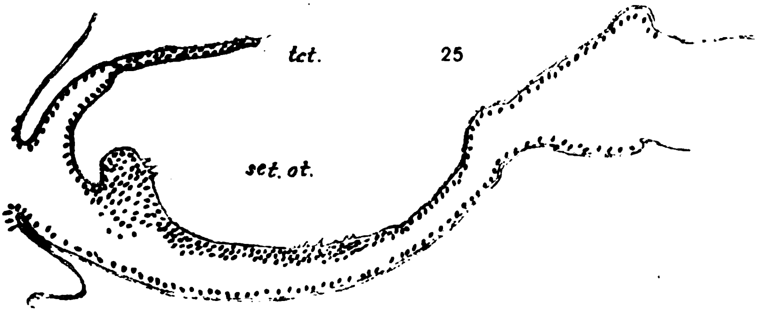

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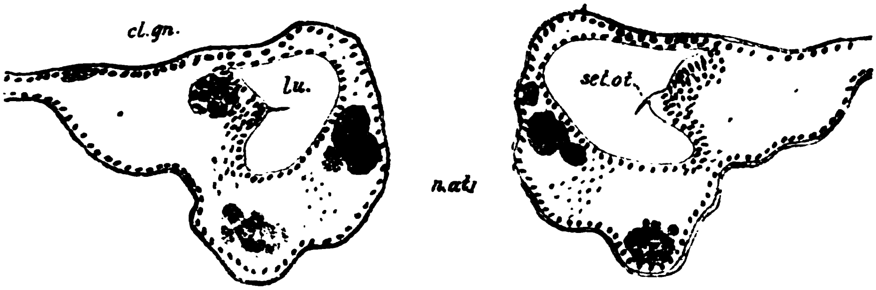
PLATE 6.

Crangon ; in Figure 28 dorsal is up ; in Figure 29 anterior is up.

Fig. 28. Transverse section of both antennules through the sensory cushions of the otocysts. × 95.

Fig. 29. Reconstruction from ten frontal sections through the base of both antennules and the brain. A semi-diagrammatic nerve element is shown at the left in black. × 95.

28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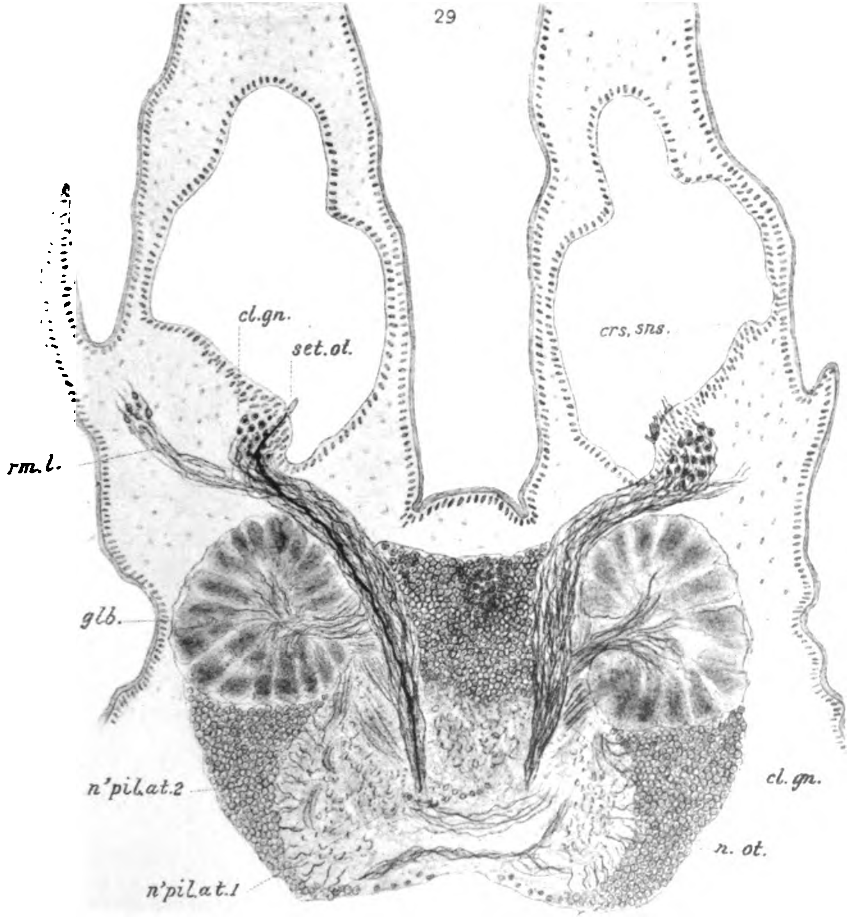
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PLATE 7.

Figures 30 and 31 are of *Crangon*. All others are of *Cambarus*. In Figure 30 dorsal is up and lateral is at the left; in Figure 36 dorsal is up and lateral is at the right.

Fig. 30.	Parasagittal section of antennule and brain. × 95.
Fig. 31.	Otocyst hair. × 600.
Fig. 32.	Olfactory hairs from basipod of second maxilla, showing innervation. Methylen blue. × 95.
Fig. 33.	Posterior row of otocyst hairs and their nerve elements. Methylen blue. × 95.
Fig. 34.	Tactile hair from scaphognathite of second maxilla, showing innervation. Methylen blue. × 95.
Fig. 35.	Transverse section through shaft of otocyst hair. × 770.
Fig. 36.	Diagrammatic transverse section of the right antennule through the posterior part of the otocyst. × 15.

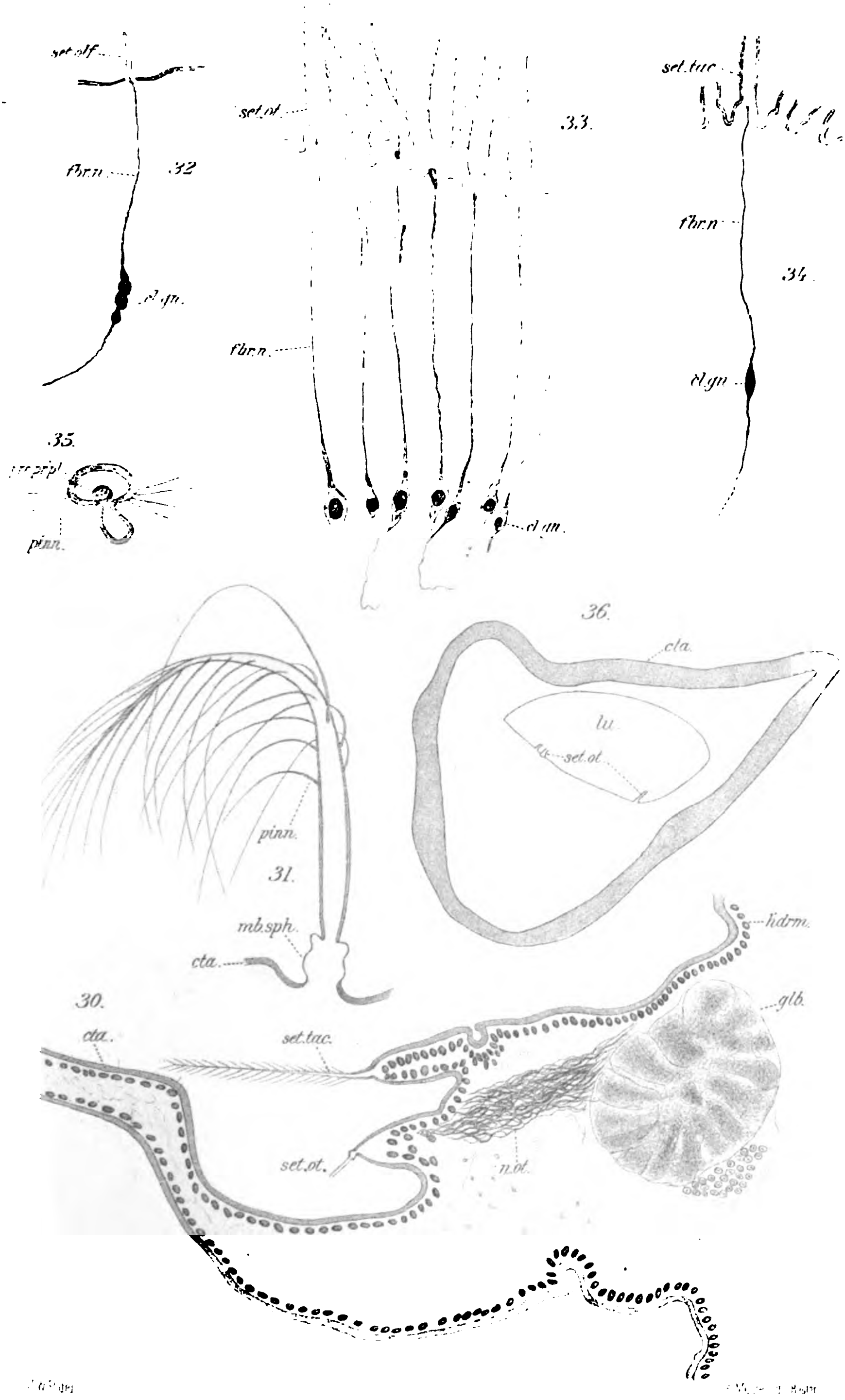
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PLATE 8.

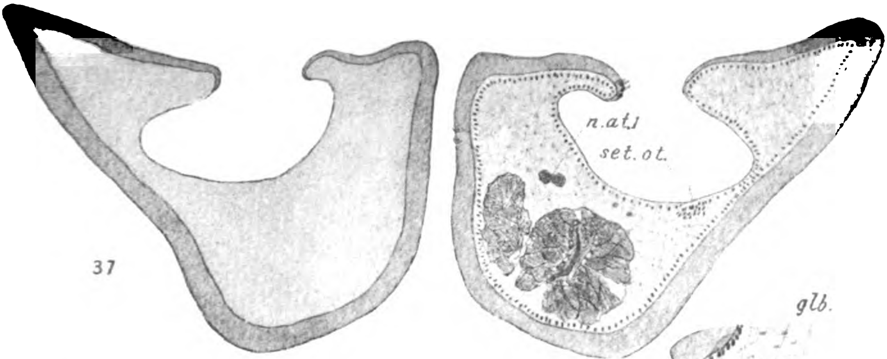
All Figures are of *Cambarus*. Dorsal side is up in Figures 37 and 38; in Figure 38 anterior is at the left; anterior is up in Figure 40.

Fig. 37. Transverse section through the orifice of both otocysts; left antennule is diagrammatic. × 25.

Fig. 38. Parasagittal section through right antennule and brain. × 25.

Fig. 39. Tegumentary gland from the sensory cushion of the otocyst. × 600.

Fig. 40. Dorsal view of the sensory cushion of the left otocyst dissected out, showing the arrangement and innervation of the hairs. Methylen blue. × 62.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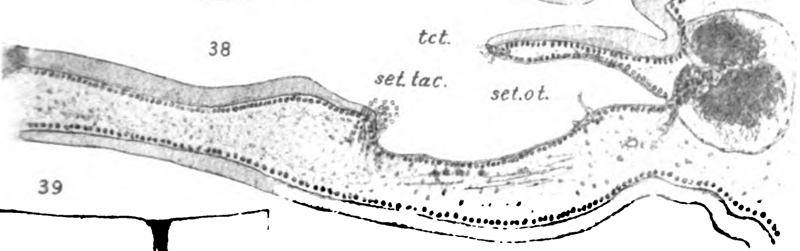
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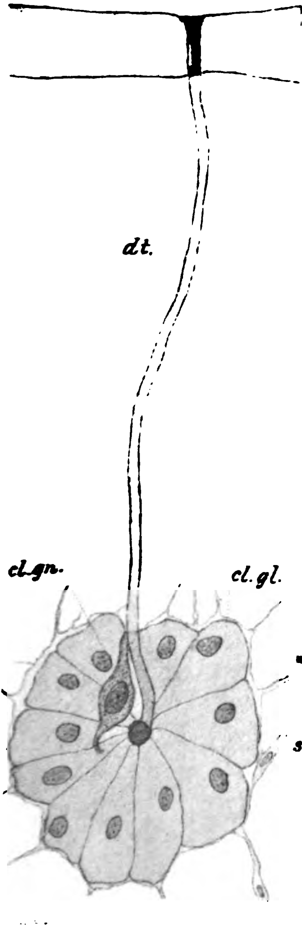
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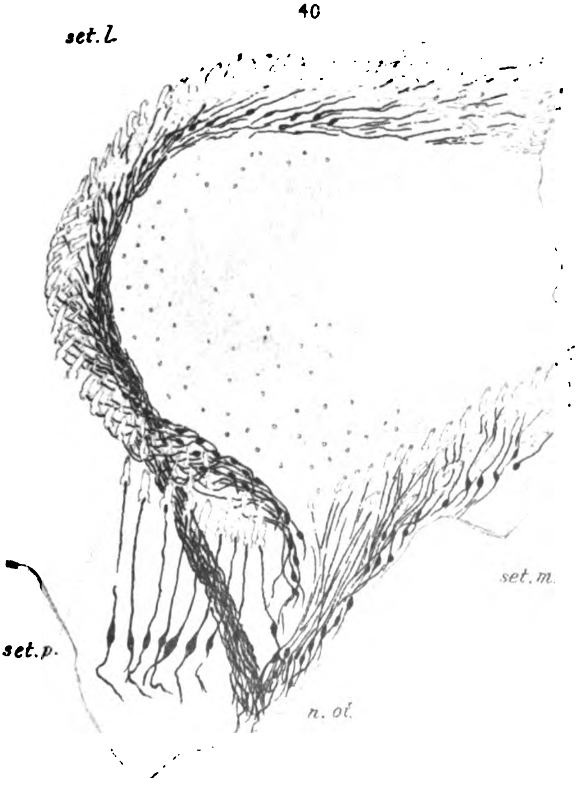
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PLATE 9.

Figure 41 is of *Cambarus*; all others are of *Carcinus*. In Figures 41 and 46 anterior is up; in Figures 42–45 dorsal is up and anterior at the left; in Figures 47 and 48 dorsal is up and lateral is at the right.

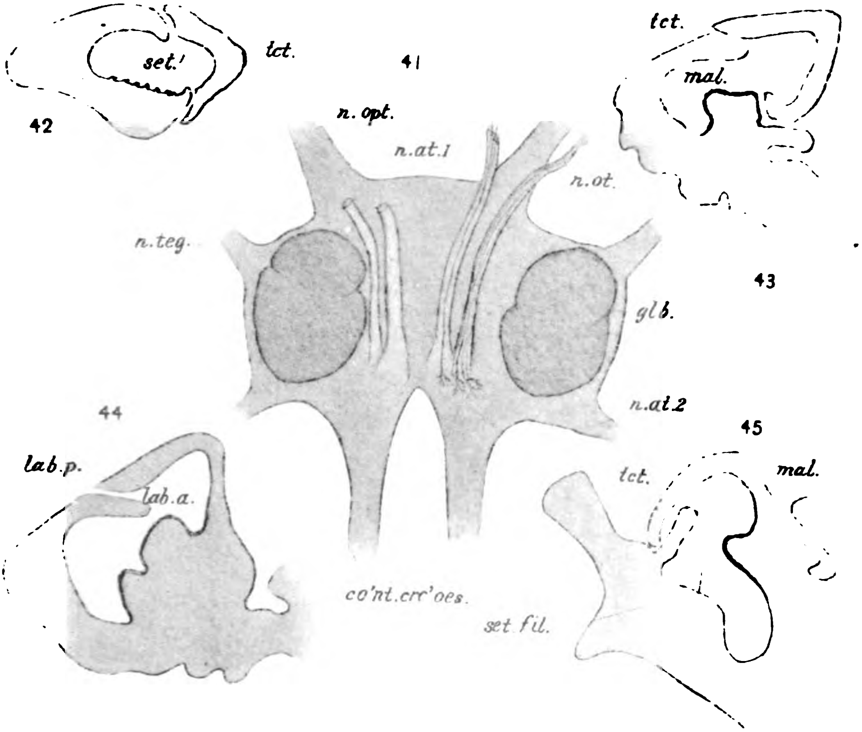
Fig. 41. Ventral view of brain, showing central endings of otic and antennular nerves. Methylen blue. × 30.

Figs. 42–45. Outlines of four parasagittal sections through the left otocyst of Carcinas, cut along the lines of section marked with corresponding numbers in Figure 46. Figure 45 is most median, Figure 42 most lateral in position. × 15.

Fig. 46. Dorsal view of both antennules. Numbered lines (*42–48*) indicate planes of section of corresponding Figures. × 8.

Fig. 47. Transverse section through the orifice of right otocyst (see Fig. 46, line *47*). × 15.

Fig. 48. Transverse section through anterior end of the otocyst (see Fig. 46, line *48*). × 15.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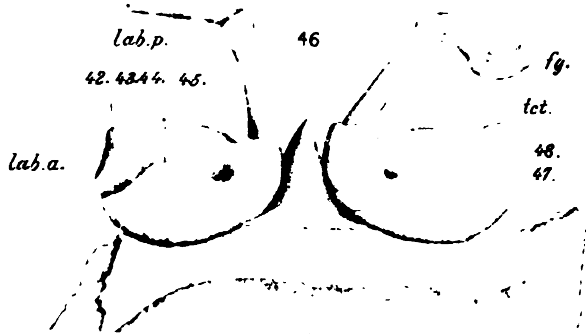
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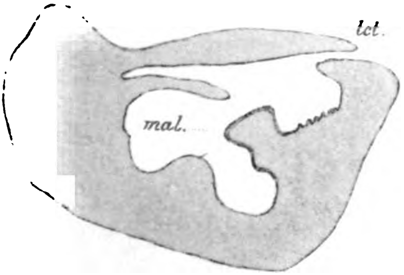
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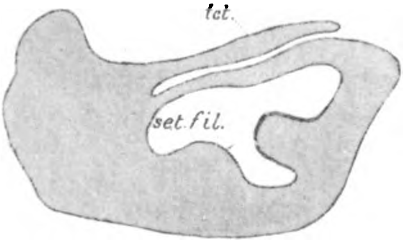
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PLATE 10.

All Figures are of *Carcinus*; anterior is up in Figure 55.

Fig. 49. Group hair. × 600.

Fig. 50. Transverse section through the sensory cushion of the hook hairs. × 168.

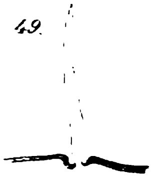
Fig. 51. Hook hair. × 600.

Fig. 52. Portion of outer flagellum of antennule, showing the bases of olfactory hairs and their innervation. × 95.

Fig. 53. Thread hairs and their nerve elements. Methylen blue. × 95.

Fig. 54. Tip of thread hair. × 1300.

Fig. 55. Nearly frontal section, inclining dorsal and forward, through both antennules and brain. × 25.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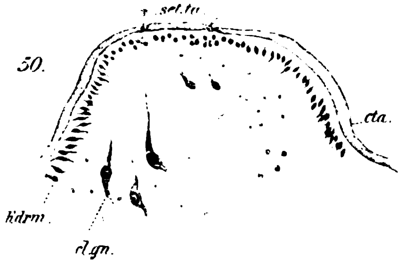
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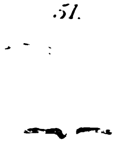
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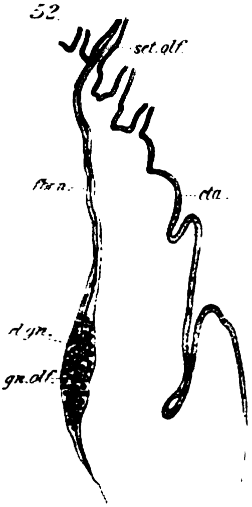
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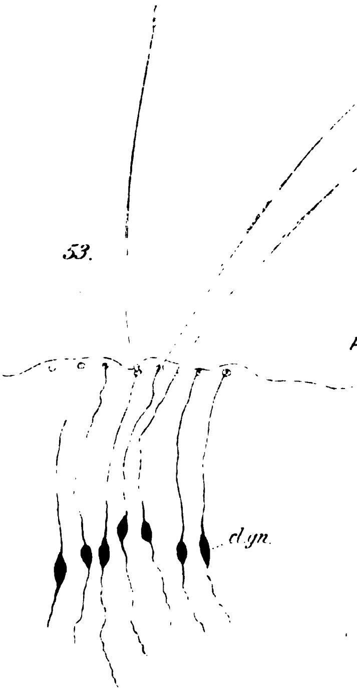
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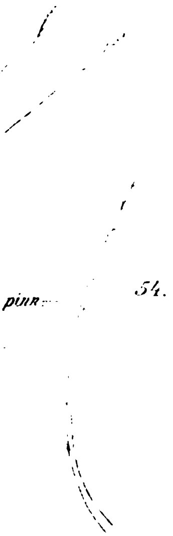
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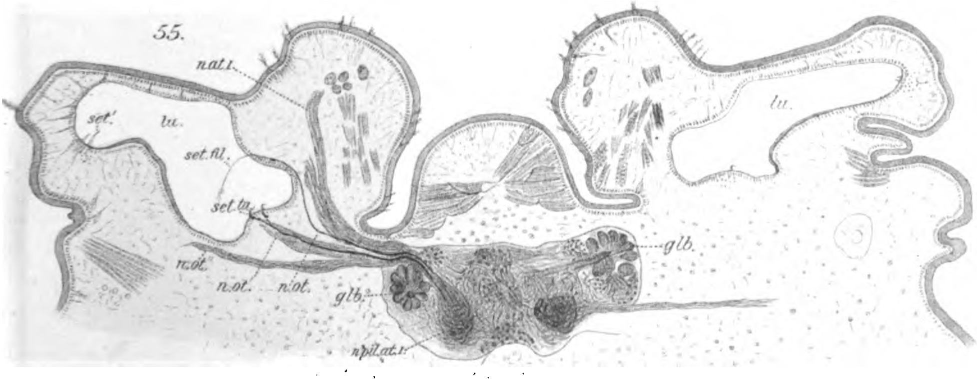
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C.W.P. del.

B. Meisel lith Boston

which take place in the formative segment and tend to restore it to the normal.

In conclusion I wish to express my thanks to Professor E. L. Mark for most helpful suggestion and criticism.

Cambridge, Mass., May 24, 1901.

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E. L. Mark, *Director*.

⁂ Abbreviations used:—

B. M. C. Z. . . . . for Bull. Mus. Comp. Zoöl.
P. A. A. . . . . for Proceed. Amer. Acad. Arts and Sci.
P. B. S. N. H. . . . . for Proceed. Bost. Soc. Nat. Hist.

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No. 124.

SOME METHODS FOR USE IN THE STUDY OF INFUSORIA.

BY A. W. PETERS.

WITH TWO FIGURES.

FROM THE AMERICAN NATURALIST,
VOL. XXXV., No. 415.

CAMBRIDGE, MASS., U. S. A.
JULY, 1901.

Bulletin of the Museum of Comparative Zoölogy
AT HARVARD COLLEGE.
VOL. XXXVII. No. 3.

THE REGENERATING NERVOUS SYSTEM OF LUMBRICIDÆ AND THE CENTROSOME OF ITS NERVE CELLS.

BY HERBERT W. RAND.

WITH EIGHT PLATES.

CAMBRIDGE, MASS., U. S. A.:
PRINTED FOR THE MUSEUM.
SEPTEMBER, 1901.

FEB 27 1902

[From the Bulletin of the Museum of Comparative Zoölogy at Harvard College, Vol. XXXVII., No. 3, September, 1901.]

No. 3.— *Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the direction of* E. L. Mark, No. 126.

THE REGENERATING NERVOUS SYSTEM OF LUMBRICIDÆ AND THE CENTROSOME OF ITS NERVE CELLS. By Herbert W. Rand.

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I. Introduction.

For several years after the description by van Beneden, in 1883, of the mitotic polar structures to which he gave the names, "sphères attractives" and "corpuscules polaires," such structures were observed only in cells undergoing mitosis, and were believed to be visibly present only during the mitotic process, although it was suggested by van Beneden himself that they might be preformed in the cell, becoming evident only during mitosis. It was therefore a matter of some interest when, in 1891, Flemming described structures in certain resting cells of the salamander similar to those found at the poles of the mitotic figure. Flemming was the first to describe these structures minutely, although the observations of Solger ('89) and Rabl ('89) had been made previously.

This discovery led to the search for the centrosome in the resting cells of various tissues, and interest in the question culminated with the announcement by von Lenhossék, in 1895, of the discovery of a centrosome and sphere in the nerve cells of the frog. Von Lenhossék was quickly followed by other authors who described similar structures in the nerve cells of other animals, and at the present time the "centrosome" is known to exist in the nerve cells of many vertebrates and invertebrates.

It is a matter of some importance, as has been pointed out by several authors, to determine whether or not the "centrosomes," "spheres," and radiations seen in the cytoplasm of fully differentiated and presumably functional nerve cells are related to the polar structures of past or future mitotic divisions of the cell. Accepting the general belief that functional nerve cells do not divide, we are limited to proving either that the centrosome and its accompanying structures of the last mitosis of the embryonic nerve cell persist as the centrosome and radiating system of the differentiated nerve cell, or that, on the contrary, they do not persist, and that we have to do in the differentiated nerve cell with structures which have arisen in the cytoplasm independently of the last mitosis of the cell. That certain parts of the radiating system of the resting cell could have been left over from the last division, and other parts not, is, of course, a possibility to be considered.

The following work was undertaken at the suggestion of Professor E. L. Mark, with the purpose of throwing some light upon the origin of the so-called centrosome of the nerve cell. The work was carried on at Harvard University under the direction of Professor Mark and Doctor W. E. Castle, from whom have been received much valuable assistance and many helpful suggestions as to the proper interpretation of the results obtained.

II. Historical.

1. The Centrosome in Nerve Cells.

Previous to von Lenhossék's discovery, in 1895, of the centrosome in the nerve cell, we find in the literature a few references to a fibrillar structure of the cytoplasm of nerve cells and a concentric arrangement about the nucleus.

Remak (**'44**, p. 469) described in the nerve cells of Astacus "sehr zarte granulirte, den Kern umkreisende Fasern." In the nerve cells of Raja he (**'53**) mentions two systems of fibrillæ, — peripheral ones extending into the axis cylinder, and deeper ones concentric to the nucleus.

Walter (**'63**), Leydig (**'64**, p. 84), Arnold (**'67**), and Schwalbe (**'68**) found a fibrillar structure concentric to the nucleus in nerve cells of vertebrates and invertebrates.

Arnold (**'65, '67**), Courvoisier (**'66**), Sigmund Mayer (**'72**), Eimer (**'77**), and other authors speak of fine fibres extending out from the nucleus, or even from the nucleolus, in vertebrate nerve cells, but from the descriptions and figures given it is impossible that these structures could have been identical with the exceedingly delicate radiations recently observed in connection with the centrosome.

Max Schultze (**'71**) found fibrillæ concentric to the nucleus in certain brain cells of Torpedo, as well as fibrillæ which enter the cell from the processes.

Hans Schultze (**'79**) described fibrillæ concentric to the nucleus in nerve cells of vertebrates.

Flemming (**'82, '82^a**) mentioned a "streifige Structur" concentric to the nucleus in the central nerve cells of the pig. In the cells of the spinal ganglion of various mammals he found a fibrillar structure, but it exhibited no concentric or radial arrangement.

Von Lenhossék (**'86**) figured and described, in the spinal-ganglion cells of the frog, an arrangement of fibrillæ generally, but not always, concentric to the nucleus. In some of his figures the nuclei are very excentrically placed and are flattened or even slightly concave on the side nearest the centre of the cell, agreeing in this respect with the conditions found in his later work on the same material, in which the centrosome and sphere were discovered.

Heidenhain ('94) declared that the presence of a centrosome was least to be expected in nerve cells. Nevertheless, he pointed out that the evidences of radial and concentric arrangement which have been described in such cells demand that a careful search for the centrosome be made.

Flemming ('95) mentioned the frequent occurrence of the concentric arrangement in the nerve cells of many animals investigated by him. He says (p. 25): "Die Anordnung ist nicht im vollen Sinne concentrisch, sondern scheint von der Polstelle in zwei opponirten Strahlungen an der Peripherie der Zelle zu verlaufen, so dass der Kern etwa die Mitte zwischen beiden bildet."

Von Lenhossék ('95), after the demonstration by Flemming and Heidenhain of the centrosome in resting cells, returned to the study of the nerve cells in which the excentric and flattened nuclei had been observed, and by methods other than those used in his previous work demonstrated the presence of a centrosome and sphere. The conditions which he finds in some of the spinal-ganglion cells of the frog are briefly as follows.

The nucleus is in a very excentric position and generally flattened, or even concave, on the side nearest the cell centre. The centrosome and sphere lie at the centre of the cytoplasmic mass, *exclusive of the nucleus;* for, as a result of planimetric measurements, von Lenhossék declares that "das Centrosom ist also in den Spinalganglien des Frosches wohl ein Centralgebilde in Bezug auf das Zellprotoplasma mit Abzug des Kerns, nicht aber in Bezug auf die kernhaltige Gesammtzelle" (p. 367). Heidenhain had found that in leucocytes the "Microcentrum" occupied the geometrical centre of the entire cell, except when displaced by a nucleus greater than one-half the diameter of the cell.

The centrosome of von Lenhossék is about 0.5 μ in diameter, and is composed of never fewer than twelve granules imbedded in a feebly staining matrix, which he compares to the "achromatische Substanzmasse" by which Heidenhain's "Centralkörper" were connected. Surrounding this group of granules is the sphere, which appears in sections as a sharply defined, homogeneous, circular area from 4 μ to 6 μ in diameter. Its sharp boundary is not due to a layer of microsomes, but is marked only by the contrast between the substance of the sphere and the surrounding cytoplasm. This contrast is often emphasized by a narrow clear area about the sphere.

The cytoplasm is arranged concentrically around the sphere, showing a differentiation into an endoplasm and an exoplasm. The endoplasm is finely granular, the granules being more concentrated toward the centre. The exoplasm gives up its stain more readily than the endoplasm, remaining diffusely stained and appearing less granular than the endoplasm.

The conditions described were found in only the smaller cells of the ganglia. No centrosome was found in the spinal-ganglion cells of the dog or cat, but a concentric arrangement of the cytoplasm about the nucleus, which in those animals was found generally at the cell centre, was observed, leading von Lenhossék to suggest (p. 368) that "das dynamische oder vielleicht auch morphologische Aequivalent des Centrosoms hier in den Kern verlagert ist." No radiations were seen by von Lenhossék. All of his figures are from preparations fixed in sublimate and stained by Heidenhain's bordeaux-iron-hæmatoxylin method.

Bühler ('95), working simultaneously with von Lenhossék, announced the discovery of "Centralkörper" in the cortex of the fore-brain of the lizard, and also in nerve cells of the human brain. Bühler used warm corrosive sublimate and Flemming's fluid as fixing agents, and stained in various aniline dyes. His best demonstration of the "Centralkörper," however, resulted from the use of Heidenhain's iron-hæmatoxylin with bordeaux, safranin, or rubin.

Bühler found the nuclei to lie generally nearer the end of the cell from which the process came off, frequently so close to the surface "dass er stellenweise die Zellgrenze zu bilden scheint" (p. 17). On the side of the nucleus toward the greatest cytoplasmic mass, and therefore opposite the nerve process where that is to be seen, appear from one to three small, intensely stained granules, lying close together, and sometimes connected by a "Substanzbrücke." About this granule or group of granules is a clearer area of cytoplasm, and there may be one or several more or less complete dark circles concentric about the central granules. Radial fibres often appear extending out from the central granules, sometimes only to the inner of the surrounding circles, and sometimes quite to the periphery of the cell. Each of these concentric circles is due to a series of varicosities on the radial fibres, at equal distances from the central granules. In some preparations, however, the radial fibres are not conspicuous, and the circles appear to be made up of rows of granules.

Other systems of fibres run more or less parallel to the cell surface, or concentric to the nucleus, and are traceable out into the processes. These fibres, which Bühler believes to be limited to the surface of the cell, also bear varicosities and appear to intersect the radial fibres, the points of intersection being marked by "kleine Knötchen." The central granules Bühler believes to correspond to the "Centralkörper" of Heidenhain, and the radial fibres to Heidenhain's "organische Radien."

As to the function of the centrosome in the resting cell, Bühler says (p. 37): "Wo, wie in reifen Ganglienzellen ihre Beziehung zur Zellteilung nicht nachgewiesen werden kann, da tritt ihre zweite Function in ihre Rechte: Sie sind in der ruhenden Zelle die Insertionsmittelpunkte für das centrirte Fibrillensystem." Bühler states (p. 38) his belief that "nicht nur das dynamische Centrum der Zellteilung in Gestalt der Centralkörper, sondern das gesammte System der organische Radien einschliesslich der Attractionssphäre dauernd in ruhenden Zellen sich erhält."

Dehler ('95) described structures in the sympathetic-ganglion cells of the frog very similar to those described by von Lenhossék for the spinal-ganglion cells of the same animal. Dehler, like von Lenhossék, obtained his best results with sublimate fixation and Heidenhain's staining methods. He found the nuclei excentric and lying at the end of the cell opposite the nerve process. They are generally flattened or concave on the side toward the cell centre. The cytoplasm contains "Schollen die sich concentrisch im Zellleib zu einander reihen." The centre about which the "Schollen" are arranged is approximately the centre of the cell. They are generally larger toward the periphery of the cell. The central region of the cell is more often quite free from them, and they are less marked, or even absent, in the smaller, cells.

At the centre of the cell lies the "Centralkörpergruppe," composed of several deeply staining granules. About them the section of the cell exhibits a well-defined circular clear area from 5 μ to 7 μ in diameter. It is very finely granular and is bounded by neither a membrane nor a row of granules. In some of Dehler's figures there is about the central clear area a considerable zone free from the coarse "Schollen." In other cases the "Schollen" extend quite to the boundary of the clear area, which corresponds to von Lenhossék's "Sphäre." Dehler found no radiations from the region of the centrosome.

Flemming ('95^a) described a concentric arrangement of granules about the nucleus of the spinal-ganglion cells of mammals, but he found no structures similar to the centrosome and sphere which then had been recently described by von Lenhossék in the frog.

Schaffer ('96) described centrosomes in certain cartilage cells of Myxine glutinosa and also in nerve cells of the cranial ganglia of Petromyzon planeri 15.5 cm. long. His material was fixed in picro-sublimate and stained in hæmalum-eosin. Schaffer found cells with the nuclei excentric. The cytoplasm was very finely granular, and in it was commonly found a structure forming "eine Art von Gegengewicht im Protoplasma gegenüber dem Kern" (p. 26). These cytoplasmic structures appeared in some cases as merely small areas of irregular shape and varying size, staining red in contrast to the surrounding blue. Often two such areas of unlike form and size lay near together. In some cases a small clear space surrounded the darker area. In still other cells a circular clear area contained a small central granule, and at one side of the clear area lay an irregularly shaped red-staining mass similar to those found where no clear area and granule were present. Schaffer interprets the granule, clear area, and red-staining mass as centrosome, sphere, and archoplasm, respectively. He attributes the failure to find all three of the structures in so many cells to unfavorable planes of cutting, or to other causes. No radial arrangement of the cytoplasm was seen. He suggests that the structures described may be concerned in nuclear divisions of the ganglion cells, since he has often seen in Petromyzon ganglion cells with two nuclei.

McClure ('96, '97) described centrosomes and spheres in the nerve cells of Helix and thinks they may exist in Limulus. In Astacus, Cambarus, Homarus, Lumbricus, Arion, and Limax he failed to find these structures. In unipolar ganglion cells of Helix with a transverse diameter between 17 μ and 22 μ he finds excentric nuclei often flattened, or more frequently kidney-shaped. His figures show the nuclei lying at the end of the cell opposite the nerve process, and the flattened or concave side of the nucleus is never "directed exactly opposite to the base of the axis-cylinder process, but always to a point on one side of it." "In the body of the cell, directly opposite the invagination [of the nucleus], a disc-shaped structure was found." The disc is finely granular, but shows no radial arrangement. "Within these discs and at about their centre, two or three small granular bodies were present which stained much deeper than the surrounding granules and which I have taken for centrosomes." A zone of cytoplasm immediately surrounding the disc stains darker than the disc, because of a concentration of small chromophilous granules, but no radial arrangement among these granules is to be seen. The centrosome and sphere were best seen in material fixed in Flemming's fluid and stained in iron-hæmatoxylin. Sublimate preparations with the same stain gave confirmatory results. In some cases, large spherical pigment granules were found arranged in a circle just outside the disc and close to its boundary.

Miss Lewis (**'96**, **'98**) described the presence of a centrosome and sphere in the giant nerve cells of Clymene producta. She demonstrated the presence of these structures by the use of vom Rath's picric-osmic-acetic-platinic chloride mixture, not followed by a stain. Sublimate fixation followed by Heidenhain's iron-hæmatoxylin gave confirmatory results.

The nucleus of these giant cells is excentric and flattened or concave on the side toward the cell centre. The sphere is a more or less sharply defined region lying near the cell centre and has a diameter about one-third that of the cell. It consists of an outer "broad zone of coarsely granular protoplasm; within this a smaller area of more nearly homogeneous protoplasm; and in the centre of this a very small highly refractive body, or occasionally two or three such bodies." The central bodies sometimes have the form of short rods. From the central granule, or granules, radiations "traverse both the inner, more homogeneous zone and the outer, coarsely granular zone. Sometimes the radiations pass even beyond this into the surrounding, finely granular protoplasm of the cell" (**'96**, p. 296). The radiations are composed of minute granules arranged in radiating lines.

Dahlgren (**'97**) examined spinal-ganglion cells of the dog and found appearances which resembled centrosomes and "centrospheres," but which he believed to be artifacts due to the crystallization of corrosive sublimate. The questionable structure, always found between the pigment mass and the nucleus, appeared in the section as a disc free from granules and having at its centre a black spot. Focussing revealed a radial arrangement of the disc, and the surrounding cytoplasm showed a "distinct concentric arrangement."

In some twenty-five cells, before the sublimate had been dissolved out by means of iodine, Dahlgren observed radiating crystals of the sublimate occupying the region where, in the same cells after staining, the supposed artifacts were seen.

That the crystals should always be found at the same region of the cell, Dahlgren says, "would indicate some difference in the constitution of the cell at this point."

Dogiel (**'97**, p. 108) mentions that, in methylen-blue preparations of the spinal-ganglion cells of certain mammals, "in einer gewissen Entfernung vom Kerne ein runder oder ovaler heller Fleck mit einem kleinen, von Methylenblau stark gefärbten Körnchen im Centrum zu bemerken war." This is probably, he thinks, the centrosome and sphere described by von Lenhossék.

Heidenhain und Cohn ('97) find the "Microcentrum" in the cells of most of the tissues of bird embryos. In the cylindrical cells of the neural tube the microcentres lie at the extreme outer ends of the cells. In a surface view of a group of these cells their outlines form a polygonal network, "dessen Maschen die übrigens nicht regelmässig central gestellten Microcentren, je eines in einem Zellenterritorium, einschliessen. . . . Findet man in einem bestimmten Zellenfelde das Microcentrum nicht vor, so ist man häufig in der Lage zu konstatiren, dass in dem zugehörigen Zellenkörper eine Theilungsfigur enthalten ist, dass somit die Centralkörper zum Zwecke der Mitose in die Tiefe der Epithelzelle eingerückt sind" (p. 205).

Bühler ('98) finds excentric nuclei in cells of the spinal ganglia of Bufo vulgaris. In the cytoplasm chromophilous flakes, which are larger toward the periphery of the cell and smaller toward the centre, are arranged in concentric order about a point lying near the cell centre. Certain fibres also participate in this concentric arrangement. Close to the nucleus and on the side toward the cell centre lie two minute granules staining deeply in hæmatoxylin and connected by a dark band. About these as a centre are one or two rows of similar but less conspicuous granules arranged in arcs of circles. Extremely fine radiations pass outward from the central granules; they intercept in their courses the granules of the concentric arcs and are sometimes capable of being followed quite to the cell periphery. Granules staining in hæmatoxylin occur at rather regular intervals along the course of a fibril. *The concentric arrangement is about the approximate cell centre and not about the granules at the centre of the radiating system.* The latter centre lies in the line passing through the centre of the nucleus and the centre of the cell as a whole, — the principal axis, as determined by Heidenhain, — but toward the nucleus end of the cell from the cell centre. The "Microcentrum" described by Heidenhain for leucocytes lay at the cell centre. Bühler says (p. 49): "Bei mehreren Centralkörpern gelang es mir fast immer, eine Verbindung in Gestalt eines dunklen Bandes, die primäre Centrodesmose Heidenhain's zu sehen." The system described by Bühler corresponds, therefore, to the "Microcentrum" and "organische Radien" of Heidenhain.

The results, then, of the work of von Lenhossék and Bühler upon the same material, the spinal-ganglion cells of the frog, are at considerable variance. They agree in showing a concentric structure in the cell body, but von Lenhossék finds the centrosome at the centre of the concentric structure, while Bühler finds his "Centralkörper" near the nucleus and remote from the centre of the concentric arrangement. Von Lenhossék's centrosome is composed of a large number of granules; Bühler finds generally only two granules composing his "Centralkörper." In the toad and other vertebrates, including mammals, Bühler finds radiations from the "Centralkörper;" von Lenhossék described no radial structures. Bühler, in the paper referred to ('98), compares critically his results with those of von Lenhossék and other authors.

Bühler states that structures similar to those described by him ('95) in the brain of the lizard he has seen also in some of von Kölliker's Weigert preparations of pyramid cells from the human brain, and likewise in Cohn's preparations of nerve cells from an adult mouse.

Hunter ('98) noticed that van Beneden et Julin ('84), in a work on the central nervous system of ascidians, represented excentric invaginated nuclei in the nerve cells of Molgula. He consequently was led to investigate the cells of the central nervous system of Cynthia partita, and found the nuclei always excentric and generally at the end of the cell opposite the process. The form of the nuclei varied from spherical to cup-shaped. Occupying the centre of the cell was a structure which he considered homologous to the centrosome and sphere of von Lenhossék. At the centre of a "clearly staining area, homogeneous or finely granular," were one or several "black deep-staining granules, the centrosome or central bodies of authors." Surrounding the clear area was a coarsely granular zone of varying diameter. In some cells were "well developed astral rays, presenting the appearance found in leucocytes."

There were many modifications of the structure. In some cells all the parts mentioned were found, while in others only a central deeply staining granule was found with no definite cytoplasmic arrangement about it. In very young animals killed shortly after metamorphosis a larger proportion of cells showed the structure than in the adults. In these young cells the sphere and radiations were generally lacking, or at most only a very narrow clear area with a slight condensation about it was found. There were generally two central granules. In the cells of the young Cynthia the nuclei were excentric but rarely invaginated, and the centrosomes seemed to have no fixed position in the cell body, often being found "laterally between the nucleus and the cell membrane." Speaking of the absence of sphere and radiations in the young cells, and the varying position of the centrosomes, Hunter says: "These facts can only be explained on the supposition that the centrosome does not exert any decided mechanical influence on the cell protoplasm."

Hunter found individual variation as to the frequency of the presence of the centrosome in the nerve cells of different animals killed at the same time, the preparations being subjected to exactly the same treatment throughout.

Rohde ('98) finds that certain "Nebennucleolen" wander out from the nuclei into the cytoplasm of nerve cells of the frog, and come to resemble closely the sphere described by Dehler. In the cytoplasm of the nerve cells of the dog he found bodies of nucleolar origin.

In nerve cells of the frog, Rohde commonly finds structures that resemble a "centrosphere." They consist of a central dark body in a clear area, which lies within a sphere exhibiting a radial arrangement. The whole structure suggests the centrosome and sphere described by von Lenhossék. Rohde, however, maintains the existence of such structures in all the cells of the ganglia, — not merely in cells of a certain size, as described by von Lenhossék, — and in any region of the cell whatever, even within the nucleus, or immediately outside the cell body. Moreover, as many as eight of them may occur in one cell. They were not found in the cells of mammals. Rohde thinks they are not artifacts. As regards the existence of the centrosome in the nerve cells of either invertebrates or vertebrates, he says, "Centrosomen kommen also bei Ganglienzellen nicht vor."

Hamaker ('98) found structures in the nerve cells of Nereis which he considered to be comparable to the centrosomes described for nerve cells by other authors. Often two or three of these structures and sometimes as many as ten were found in a single cell. Each one consisted of a deeply stained granule in a clear space enclosed within a hollow sphere of coarse granules. No radiations were seen. The nuclei were excentric and often flattened. The "centrosomes" lay at the centre of the cell body.

Holmgren ('99) occasionally finds sections of cells which exhibit a central structure very similar to the sphere of von Lenhossék. He describes a system of fibres which sweep in from the periphery of the cell to form a "Spiralfigur." Similar conditions are described by Bühler ('98). Among the generally hyaline fibres of this system are some more deeply staining fibres. Holmgren thinks certain favorable sections through this spiral system give the appearances interpreted by von Lenhossék as a sphere, his "centrosomes" being simply sections of some of the deeply stained fibres that happened to lie at the centre of the spiral figure.

Kolster (**'00**) gives a brief preliminary account of centrosomes in the nerve cells of Cottus scorpius. The only two figures given represent one or two deeply stained granules surrounded by a small clear area and lying near the cell centre, the nuclei being excentric and concave on the side toward the cell centre.

2. The Centrosome in Other Resting Cells.

Leaving nerve cells and turning now to the question of the centrosome in other resting cells, and especially in tissue cells, a considerable literature on this subject is found to have grown up during the last ten years. Previous to 1889 no accurate description had been given of a centrosome and sphere in resting tissue cells, but doubtless many of the descriptions of "Nebenkerne" and "Centralmassen" refer to structures that would now be recognized as centrosomes or spheres.

Van Beneden et Neyt ('**87**, p. 279) found that the attraction sphere persisted during the resting periods of the early cleavages of Ascaris. "Nous sommes donc autorisés à penser que la sphère attractive avec son corpuscule central constitue un organe permanent, non seulement pour les premiers blastomères, mais pour toute cellule; qu'elle constitue un organe de la cellule au même titre que le noyau lui-même; que tout corpuscule central dérive d'un corpuscule antérieur; que toute sphère procède d'une sphère antérieure, et que la division de la sphère précède celle du noyau cellulaire."

Boveri (**'88**) showed the continuity of the centrosome in the cleavage of Ascaris, and set forth his conception of a specific "archoplasm," which is strewn through the cell in granular form during the resting period, and, in mitosis, collects about the centrosomes to give rise to the radiating systems.

Rabl (**'89**) observed that the resting nuclei of epithelial cells in Triton were indented upon one side, while in the cytoplasm, in the region of the indentation, was a highly refractive homogeneous mass which, he suggested, was due to the presence of the "Polkörperchen" or the "Attractionssphäre."

Solger (**'89, '90**) found a radiating structure in the pigment cells of some teleosts. The cells frequently have two nuclei which are situated at the cell centre, and between them is a small clear area, from which proceed radiating lines of granules toward the periphery. The central area Solger suggests to be "das Centralkörperchen oder die Sphère attractive im Ganzen oder ein Theil derselben."

Kölliker ('89) found the attraction sphere in cleavage cells of Siredon while the nucleus was in the resting condition.

Bürger ('91) found an attraction sphere with centrosome and radiations in the cells of the body fluid of nemertines.

Flemming ('91, '91[a], '91[b]) described "die strahligen Sphären und ihre Centralkörper" in resting leucocytes of the salamander. In the amitotic divisions of the nuclei of these cells the sphere does not divide, but Flemming believes that it exerts some influence over the amitotic process, since "bei den Abschnürungen die Sphäre eben nicht einer beliebigen Stelle der Kernmasse benachbart liegt, sondern grade an den Abschnürungsbrücken" ('91[a] p. 285). The centrosome was also found in the resting cells of the lung epithelium and in the flat connective-tissue cells and endothelial cells of the peritoneum of the salamander larva, but in these fixed cells no radiations could be seen. The "Centralkörper" generally consisted of two minute granules lying close together in the cytoplasm very near the nuclear membrane. Often a dark band could be seen connecting the two granules. When the nucleus was approaching the spireme condition the centrosomes were farther apart and this dark band was more conspicuous. Flemming therefore believed it to be "die erste Bildungsanlage der Spindel."

Flemming's discovery, made on leucocytes of the salamander, was corroborated by Heidenhain ('91), who also found the centrosome and sphere in the cells of the bone-marrow of young rabbits, as well as in pathological epithelium from human lungs and in some leucocytes from the same human material.

Henneguy ('91) showed the continuity of the attraction spheres in the developing trout egg.

Hermann ('91) found an "Archoplasmastrahlung" in resting spermatogonia of the frog, and resting spermatocytes of Helix pomatia. In resting spermatocytes of Proteus he describes an archoplasm with a conspicuous central granule.

Meves ('91) described amitosis in the spermatogonia of the salamander. During the dividing of the nucleus the "attraction sphere" took the form of a ring about the region of constriction.

Solger ('91) described an attraction sphere in resting chromatophores of teleosts.

Heidenhain ('92) observed, in two cases of leucocytes with two nuclei, two spheres present, with a well-developed spindle formed between them.

Generally the leucocytes had a single sphere, even though more than one nucleus may have been present.

Vom Rath ('92) found amitotic conditions in the "Stützzellen" of the testis of Gryllotalpa. He says, "Es gelang mir mehrfach in unmittelbarer Nähe der Kerne zwei winzige Centrosomen zu erkennen und ebenso constatirte ich in seltenen Fällen bei eingeschnürten Kernen eine deutliche Strahlung um die beiden Centrosomen, die offenbar als Attractionssphäre bezeichnet werden muss" (p. 115).

Van der Stricht ('92) showed the continuity of the attraction spheres in the cleavage of Triton. He also showed that the centrosomes of mitosis in cartilage cells of various vertebrates persist in the resting cell.

Brauer ('93, '93ª) discovered the centrosome *within* the resting nucleus of spermatocytes of Ascaris megalocephala univalens. Preceding a division of the cell, the centrosome divided while inside the nucleus and the two resulting centrosomes migrated into the cytoplasm.

Moore ('93) described an archoplasm with a "central body," "medullary zone" and radiations in resting cells of the undifferentiated genital ridge of the larval salamander. The archoplasm gave rise to the spindle of mitosis. In leucocytes of the larval salamander a similar structure was found, instead of the simpler conditions described by Flemming ('91).

Vom Rath ('93) described attraction spheres and centrosomes in various resting cells of Amphibia and studied the behavior of these structures during amitosis of the sexual cells. The structures were found also in the sexual cells of Astacus.

Watasé ('93, p. 442) concludes that "the *centrosome* is not a unique organ of the cell, but is identical with the *microsome* which exists everywhere in the cytoplasm."

Zimmermann ('93) described what he considered to be a modified attraction sphere in pigment cells of teleosts. There is a very much elongated "Centralstab," comparable to a centrosome, imbedded in a correspondingly elongated "Archiplasm," whence proceed radiations. In certain very large cells the radiations proceed from a "Centralnetz" instead of from a "Centralstab." In the smaller cells a spherical "Archiplasm" with radiations and a minute centrosome were present.

Heidenhain ('94) investigated, in great detail, the conditions in leucocytes and other cells of vertebrates. The "Microcentrum," which he finds generally present, consists of two or three granules imbedded in an achromatic "Centrodesmose," which he believes gives rise to the spindle. The microcentre is the point of insertion of a system of radiating fibres, which extend to the periphery of the cell. These fibres, according to Heidenhain, are contractile and in a state of tension, which is a source of energy displayed in the mitotic processes. The microcentre lies at the centre of an astrosphere, which is bounded by a layer of microsomes occurring on the radial fibres at equal distances from the centre. The astrosphere is not an organ of the cell, but only a region which takes a characteristic stain because of the concentration of the radial fibres.

In the giant cells of bone-marrow, which have polymorphic nuclei, mitosis occurs without a division of the cell body. In these cells several groups of "Centralkörper" were found, each group containing many granules.

Heidenhain predicted that the microcentre with its radiating system would be found in most resting cells.

Reinke ('94) found the centrosome and sphere in leucocytes of the salamander. Leucocytes of amœboid form differed from those of resting form in having coarser radiations of unequal thickness and thickenings of the fibres arranged in arcs concentric about the centrosome.

Dehler ('95ᵃ) described a microcentre (Heidenhain), without radiations, in the red blood corpuscles of the chick embryo. He believed the microcentres to be derived from the centrosomes of mitosis.

Drüner ('95) observed centrosomes in the resting sperm cells of the salamander and in resting cells of the gastrula of Triton alpestris. He gives an extended criticism of Heidenhain's mechanical theory of the centred system and sets forth an opposed theory. Drüner believes that the radiations of the resting cell disappear before a mitosis and that new radiations arise from the centrosomes. He divides the mitotic process into two periods, the first ending with the division of the chromosomes in the equatorial plate. "Die erste Periode ist die der Expansion, die zweite die der Kontraction des gesammten Strahlensystems" (p. 333).

Niessing ('95) found the centrosome with sphere and radiations in the liver and spleen cells of the salamander and in the liver of the human embryo.

Vom Rath ('95ᵃ) described a centrosome and sphere in the large gland cells of the head of Anilocra mediterranea; also in the hepatopancreas cells of Porcellio scaber and in spleen cells of a young dog. In amitotically dividing leucocytes and sperm cells there are sometimes one and sometimes two centrosomes and spheres. Vom Rath concludes "dass eine Theilung der Attractionssphäre bei der Amitose stattfinden kann . . .; ob sie aber immer stattfinden muss, ist unwahrscheinlich" (p. 61).

Von Erlanger (**'96**) observed the centrosome in resting epithelial cells of salamander larvæ. There were always two, connected by a band of substance. In mitosis these centrosomes moved apart and the spindle formed between them. In another paper (**'96**^{a}) he describes a "Nebenkern" in resting sperm cells of the earthworm. In rare cases a central granule and weak radiations were present. He believed the structure to be archoplasm and centrosome.

Meves (**'96**), in the spermatogonia of the salamander, found the spheres of adjacent cells connected by bands of substance which are probably remnants of the spindle.

Ballowitz (**'97**^{a}) demonstrated the centrosome and sphere in the cells of the pharyngeal and cloacal epithelium of Salpa, without the use of stains. The material was fixed in either weak or strong Flemming's fluid, sublimate, or acetic-sublimate, and examined in water. The nuclei are sickle-shaped, and in the concavity of each could be seen the large sphere which Ballowitz had previously described (**'97**). A detailed study of the epithelial cells of Salpa appeared in a later paper (**'98**^{a}). The centrosomes were best seen in the unstained Flemming's preparations. At the centre of the sphere were generally two, but sometimes three or four, very highly refractive bodies. They often appeared to be irregularly shaped or elongated, instead of spherical granules. Ballowitz concludes "dass es zum Nachweise der Centrosomen nicht immer einer specifischen Färbung bedarf, dass diese wichtigen Zellbestandtheile vielmehr auch in ungefärbtem Zustande in Folge ihres charakteristischen starken Lichtbrechungsvermögens so sharf begrenzt hervortreten, dass sie leicht und sicher in der Sphäre erkannt und unterschieden werden können" (p. 358).

Ballowitz believes the sphere and centrosomes to be present also in the epidermal cells of Amphioxus larvæ (**'98**). These cells have nuclei varying in form from those that are deeply invaginated to those that are sickle-shaped or complete rings.

Eisen (**'97**) described a new element found by him in the blood of some amphibians, reptiles, and man. To this element he gave the name of "plasmocyte." He attempts to show that the plasmocytes "are composed of the centrosomes and archoplasm (with part of the cytoplasm) of the nucleated erythrocytes, . . . surviving in the blood serum as free and independent elements capable of growth through assimilation of food, and taking their place as blood elements, equal in importance to the erythrocytes and leucocytes" (p. 13).

Von Lenhossék (**'98**), in studying the development of the spermatozoa of the rat, found the flagellum to be a product of the centrosome. In another paper (**'98**^{a}) he described the centrosomes and "Basalkörperchen" in the cells of ciliated epithelium. In cells that did not bear cilia, the microcentre was present, lying at the extreme outer end of the columnar cell. In the ciliated cells no microcentres were found, but there was a layer of granules just beneath the ciliated surface of the cell, the number of granules corresponding closely with the number of cilia. Von Lenhossék gives the name of "Basalkörperchen" to these granules, and thinks they are homologous with the microcentre of the non-ciliated cells.

Zimmermann (**'98**) discusses the centrosome in epithelial and gland cells of mammals, including man. He points out that, as in mitosis the centrosome is the centre of motor processes, so in resting cells the centrosome is always located at the centre of activity. If there is an equal degree of activity throughout the cell, the centrosome is at the centre of the cell, unless displaced by a large nucleus (leucocytes, pigment cells, non-striated muscle cells). In gland cells, it is at the centre of secretory activity. In ciliated cells and epithelial cells with pseudopodial processes, the microcentre is close to the outer end of the cell. Where there is a single flagellum, as in the spermatozoön, the centrosome is at the point where the flagellum enters the cell. He says (p. 697): "Ich glaube . . . dass, ganz allgemein gesprochen, das Mikrocentrum das motorische Centrum, also das 'Kinocentrum' der Zelle sei (gegenüber dem Kern als 'Chemocentrum')."

Von Lenhossék (**'99**) describes a microcentre, in the sense of Heidenhain, in the non-striated muscle cells of the cat. As non-striated muscle cells are known to divide by mitosis in cases of regeneration, we should expect to find the microcentre in the resting cell.

Among other authors who have described centrosomes in resting cells may be mentioned Hansemann (**'91**, **'93**; cells of human brain tumor, cancer cells, human leucocytes, mesenterial connective tissue of young cats and rabbits); Guignard (**'91**, **'97**; plant cells); Eismond (**'94**; cleavage cells of Triton tæniatus and Siredon); Zimmermann (**'94**; human epithelium, stroma cells of cat ovary); de Bruyne (**'95**; connective-tissue cells); Meves (**'95**; spermatogonia of salamander; **'95**^{a}; sesamoid cartilage cells in the tendon achilles of the frog); Moore (**'95**; sperm cells of elasmobranchs); Rawitz (**'95**; sperm cells of salamander); Gulland ('96; leucocytes); Spuler ('96; connective-tissue cells); Child ('97; ovarian stroma cells of mammals); Heidenhain ('97; red blood corpuscles of duck embryo); Rawitz ('98; sperm cells of Scyllium canicula); Niessing ('99; lymphocytes).

Reviews of the centrosome question, with lists of literature, have been given from time to time by various authors, among them Flemming ('93), Moore ('93), Häcker ('94), Heidenhain ('94), vom Rath ('95), Henneguy ('96), von Erlanger ('97), Heidenhain ('97), Kostanecki und Siedlecki ('97), Bühler ('98; centrosome in nerve cells), and Meves ('98).

III. Methods of Investigation.

The identity of the so-called centrosome of nerve cells with the centrosome of mitosis can be established by a series of observations showing that the centrosome of the last mitosis of the embryonic nerve cell persists through the development of the nerve cell and becomes the structure seen in the resting cell; or, if the cells can be induced to divide by mitosis, it may be possible to show that the centrosome of the resting cell gives rise to the centrosomes of the mitotic figure. The problem, then, is open to attack from two directions. Given, material in which the presence of a centrosome in the differentiated nerve cells can be demonstrated, — as it has been in many vertebrates and invertebrates, — and in which the centrosome is also to be found in the embryonic neural tissues, — as Heidenhain und Cohn ('97) have found it in the neural epithelium of bird embryos, — then we may seek to establish the genetic relationship of the organ in the embryonic cell with that of the differentiated cell.

A second method of investigation is offered by the process of regeneration. If any cells of adult nervous structures are capable of undergoing mitotic division, regeneration would seem to offer the most favorable opportunity for the exercise of such power. If, however, the regenerated nerve cells do not arise from old nervous tissues, the development of the new nerve cells from other tissues would still offer opportunities for determining whether the "centrosome," if present in the regenerated cells, had persisted from the last mitosis in the history of the cell.

The question arises as to whether processes which take place in the *embryonic* origin of nerve cells will be repeated in the *regenerative* development of similar cells. To show that a centrosome in a regenerated nerve cell is one of the centrosomes of the mitosis of the mother cell, does not necessarily prove that the centrosome of a nerve cell which has arisen by the natural embryonic process of development is also identical with a centrosome of the mitosis of its parent cell. Yet, if the regenerated nerve cells arise from ectodermal tissues, we should certainly expect the processes of regenerative development to resemble very closely those of embryonic development, especially in so fundamental a thing as the fate of mitotic organs. Many investigations have shown the similarity of regenerative and embryonic processes. If it is shown that the "centrosome" of a regenerated nerve cell is a true centrosome, — that is, the organ concerned in mitosis, — it must be admitted as highly probable that the similar organ in the naturally developed cell is likewise a true centrosome.

In an investigation upon any one kind of material, the regenerative process has an advantage over the embryonic, for it affords not only the opportunity for the study of the development of nerve cells, but also the possibility of observing whether the structures under consideration in the already differentiated nerve cells have anything to do with the formation of new cells.

The observations about to be described have been made upon tissues in process of regeneration, the object being twofold, — to determine, first, the behavior of elements in old nervous structures in the presence of the necessity for regeneration; and, secondly, the source and method of development of the new nervous parts.

1. MATERIAL.

Two qualities were demanded of material intended for this work. The fully differentiated nerve cells must contain some such structure as has been called a centrosome by various authors, and the animal must have the ability to regenerate excised parts of the nervous system. It was at first proposed to work upon the annelid, Axiothea (Clymenella) torquata. In the giant cells of a nearly allied form, Clymene producta, Miss Lewis ('96, '98) found the centrosome and sphere to be present. I found some evidence of a centrosome in the giant cells of Axiothea, but nothing as definite as Miss Lewis demonstrated for Clymene. Axiothea was found to regenerate segments very readily at either the anterior or posterior end. I obtained regeneration in a large number of the worms during the summer of 1898, at the laboratory of the United States Fish Commission at Wood's Hole. A number of segments having been cut from either or both ends, the worms were placed in glass vessels partly filled with clean sand over which ran sea water. In the course of two to four weeks a considerable regeneration was found to have taken place at the cut ends. Two facts caused this material to be abandoned. The centrosome in the giant cells was too indefinite a structure to deal with satisfactorily, while the very limited number of giant cells in a segment made the prospect of obtaining a comprehensive series of conditions in the regenerative development of these cells anything but encouraging.

Hamaker ('98) demonstrated some centrosome-like structures in the nerve cells of Nereis. I made preparations of Nereis and found some very decided evidence of the presence of the centrosome in the cells of the ventral ganglia (page 115). At the same time I examined nerve cells of the earthworm, obtaining results which inspired further investigation of that material. This fact, together with the well-known regenerative power of the earthworm and the greater ease of conducting regeneration experiments upon the land annelid, determined its use for this work in preference to Nereis. It is, therefore, mainly with the earthworm that the following work has to do.

2. Regeneration Methods.

The worms, Allolobophora (terrestris Savigny?) and Lumbricus agricola Hoffmeister, were easily obtained near the laboratory in Cambridge. From five to ten segments were removed from the anterior end, and the beheaded worms were placed in large earthen jars filled with the soil in which the worms were found. The soil was first carefully examined and all other worms removed. Most of the regeneration was obtained during the winter or early spring months. The jars were kept in the vivarium at a temperature of about 16° C. The earth was moistened from time to time so that it never became dry at the surface. Many of the beheaded worms burrowed a short distance below the surface, but many others refused to burrow and persisted, if buried under a little earth, in returning to the surface. To protect them from the light and from drying, sheets of moistened filter paper were spread over the surface of the earth and the jars were covered with glass plates.

The cutting of the anterior end prevented feeding. In the course of a few days the intestine of the worm was entirely free from earthy material. The smaller worm, Allolobophora, generally burrowed beneath the surface and coiled itself into an intricate close knot, remaining in that condition, unless disturbed, as long as it was kept, — five or six weeks. The larger earthworm, Lumbricus, which generally remained on the surface, was always found stretched out when the earth was exposed for moistening.

An attempt was made to keep some worms during regeneration in glass vessels containing moist filter paper, instead of in earth; but the method was more troublesome and possessed no advantages over the other. Unless the paper was changed every day or two, the worms were likely to die, while of those kept in the earth only about ten per cent died during regeneration.

Worms were killed after from seven to forty days subsequent to the operation. In Allolobophora, the light-colored conical bud that marked the regenerated segments was usually to be seen in two or three weeks after the operation of cutting. Lumbricus regenerated much more slowly. All of the preparations which I shall discuss are from individuals of Allolobophora.

Regeneration of posterior ends was also obtained, but little was done upon that material. The anterior ends have the advantage that the regeneration of both the brain and ventral nerve-cord can be observed.

When the desired period of regeneration had elapsed, the worms were dropped into fresh water for a moment to remove the clinging earth. The anterior end, including some six to twelve of the old segments, was clipped off with scissors and the fragment bearing the regenerated part was at once dropped into the fixing fluid. Stupefaction, where such small fragments were to be fixed, was found to be of no advantage.

In the less advanced stages of regeneration the inability to feed made unnecessary the cleaning out of the intestine before fixing. In some of the more advanced stages feeding had been resumed and the worms had to be kept upon moist linen for a day or two until the anterior part of the intestine had become clean.

3. Fixing Fluids.

The best fixing fluid for general purposes was found to be Flemming's stronger chromic-osmic-acetic mixture. This fluid not only gave the best fixation of the old ganglion cells, but also gave by far the most satisfactory fixation of mitotic cells. Some of the best demonstrations of the exceedingly fine radiating fibrillæ in the ganglion cells were obtained by this method, and the achromatic fibres of the mitotic figure were very sharply brought out.

Material was left in the fixing fluid for from five to forty-eight hours. A longer or shorter time within these limits gave no appreciable difference in results. The objects were then washed in distilled water for about twenty-four hours, then passed through ascending grades of alcohol, cleared in cedar oil and imbedded in paraffin.

Hermann's acetic-osmic-platinic chloride mixture was used with good results, although it seemed to be in no way superior to the Flemming mixture. The Flemming preparations gave a rather better quality of stain with Heidenhain's iron-hæmatoxylin. The method of treatment with Hermann's fluid was similar to that just described for Flemming's fluid.

Vom Rath's picric-osmic-acetic-platinic chloride gave no results of value. The after treatment with crude pyroligneous acid was used as described by vom Rath ('95). In some cases a two per cent solution of pyrogallic acid was substituted for the pyroligneous, with similar results. The length of time of the after-treatment, during which the reduction of the platinic chloride takes place, was varied, but in all cases the cytoplasm was blackened to such an extent that it became too opaque for the observation of the exceedingly delicate structures revealed by other methods. In some preparations mitotic figures were brought out with remarkable clearness by this method, especially as to the sharpness of spindle fibres, but, for the observation of polar and cytoplasmic structures, fixation in Flemming's or Hermann's fluid followed by a regressive stain gave results far superior to those obtained by a method which depends upon the deposition of a metallic salt in the cell structures.

Corrosive sublimate in a saturated aqueous solution containing one per cent of acetic acid was used with results which corroborated those obtained by the Flemming and Hermann fluids as to cytoplasmic structures in the ganglion cells, but the demonstration of these structures was far inferior to that obtained by the other methods. The sublimate failed to bring out the finer structure of the cytoplasm as clearly as the osmic mixtures. Radiating fibres were very indistinctly shown. The sublimate gave decidedly inferior results in the demonstration of mitotic figures. My preparations lead me to believe that the sublimate effects violent mechanical injuries in delicate tissues. In such solid tissues as the epidermis or intestinal epithelium there were no evidences of injurious effects; but among the very loosely aggregated masses of cells that constitute some of the regenerated parts, there were signs of serious damage. In the epidermis, for example, spindle figures occur imbedded in a dense cytoplasmic mass. These figures were faithfully preserved by the sublimate. In the regenerating brains and ganglia, the mitotic figures more often occur in cells the remaining contents of which appear to have become entirely fluid. Such figures, unsupported by a dense cytoplasm and completely exposed to the action of the fixing fluid, were very seriously distorted in the sublimate preparations. In all cases spindle fibres were very poorly shown in the sublimate preparations.

As for the superiority of Flemming's fluid for the demonstration of the centrosome, my experience agrees with that of Ballowitz ('97ª, p. 358) who demonstrated centrosomes in epithelial cells of Salpa without the use of stains. He succeeded in doing this with sublimate, but Flemming's fluid gave by far the best results. He says, "Ich behaupte auf Grund meiner Erfahrungen an meinem Untersuchungensobject, dass es mit grösserer Sicherheit und mehr Constanz gelingt, die Centrosomen an dem mit Flemming'scher Lösung fixirten, ungefärbten Material zu erkennen, als durch specifische Tinction an den mit Sublimat behandelten Objecten sichtbar zu machen."

My Flemming preparations were all stained with iron-hæmatoxylin, but they were decolorized to such an extent that, examined by a low power, the cell bodies appeared scarcely darker than in the unstained preparation. But examination with high power revealed the fact that the cell granules had retained the stain. It was in such preparations that central granules were most clearly seen, because of their deep stain in contrast to the unstained ground substance of the cytoplasm. Hermann's fluid gives substantially the same results in this respect. I have examined unstained Hermann's fluid preparations and have been able to distinguish fairly well the granules and fibrillæ of the cytoplasm. In stained sublimate preparations there is apt to be present in the cytoplasm a large amount of deeply staining material scattered about in irregular masses, obscuring finer details of structure. If the decolorizing is carried so far as to clear out these masses, the granules and fibrillæ are left much less distinct than in the decolorized osmic acid preparations.

All the material was cleared in cedar oil and imbedded in paraffin. Sections were cut generally 6⅔ μ thick. A few series were cut at 3⅓ μ.

4. Stains.

Heidenhain's iron-hæmatoxylin proved to be by far the most useful stain. Sections were treated with a two per cent solution of iron-alum from three-quarters of an hour to three hours and stained about the same length of time in a one-half per cent solution of hæmatoxylin. Decolorization was effected by the two per cent mordant, the process being controlled under the microscope.

This stain gave the best results both for the structure of the nerve cells and for mitotic figures in the regenerating tissues. No advantage was found to be gained by using safranin in combination with the hæmatoxylin. From what has been said, then, it follows that fixation in Flemming's mixture, followed by the iron-hæmatoxylin stain, proved to be the best combination.

Gentian violet was used to obtain sharp selection of kinetic chromatin, but it was useless for finer cytoplasmic structure. Kernschwartz gave results similar to those obtained by the iron-hæmatoxylin, but inferior in clearness. One sublimate series was stained in Kernschwartz and safranin with fairly good results, especially as to some cells of the regenerated epidermis (Figures 44 and 45).

Whatever the stain used, the best demonstration of the "centrosome" and cytoplasmic fibrillæ was obtained when the stain had been well extracted from the cytoplasm. In heavily stained cells the finer structures were made out with much greater difficulty.

5. DRAWINGS.

The preparations were studied with a Zeiss $\frac{1}{18}$ oil immersion and Zeiss achromatic oculars 3 and 4. The drawings were made with the aid of an Abbé camera. Attention is called to the fact that all the drawings of cells and mitotic figures are to the same scale, a magnification of 2000 diameters. The outlines of cells, nuclei, and nucleoli were traced with the aid of the camera. Generally the "centrosome" and prominent granules could also be located, but only in rare cases could the radiations represented in the figures be seen with the camera in place. The figures are reproduced from pencil drawings.

A diagrammatic treatment of the drawings was avoided as far as possible, and it was attempted rather to reproduce, as accurately as could be, the appearances seen in the preparations. The drawings were reproduced by the heliotype (gelatine) process because by it a more accurate reproduction of the relative conspicuousness of the structures represented in the drawings may be obtained than by the lithographic process. It must be understood, however, that in the figures of nerve cells the conspicuousness of the radiating systems is slightly exaggerated. These lines catch the eye more readily in the printed figures than they do in the preparations. In most cases it required the most careful focussing and varying of illumination to bring out the less distinct radiating fibrillæ.

IV. Observations.

1. The Nerve Cells of Lumbricidæ.

My first study to ascertain the condition of the nerve cells of the earthworm was made upon normal unregenerated material fixed in sublimate and stained in iron-hæmatoxylin. Some evidences of concentric and radial cytoplasmic structure were found, suggesting the presence of structures which might be brought out more clearly by better methods.

In transverse sections of the ventral ganglia the greater number of ganglion cells are cut in planes parallel to their long axes. The cells are situated ventrally and laterally in the cord. They are unipolar and pear-shaped, the larger ends being peripheral, while the processes extend dorsally and centrally. The nuclei, with extremely rare exceptions, are situated in the process end of the cell, often so far toward that end that the nuclear membrane is tangent to the tapering sides of the cell. The diameter of the nuclei varies from 6 μ to 16 μ. The cells are from 22 μ to 60 μ in length, and from 10 μ to 28 μ in transverse diameter.

The nuclei are always nearly or quite spherical and *never invaginated.* In the resting nerve cells of many animals where the centrosome has been found the nucleus has been described as invaginated, the centrosome occurring in the cytoplasm on the invaginated side of the nucleus. The position of the nuclei in the nerve cells of the earthworm is such as to leave by far the greater mass of cytoplasm on that side of the nucleus opposite the nerve process.

Early in my study of these cells I was impressed with the fact that, in a large number of them, more or less irregular small masses of deeply stained material occurred near the nucleus and on the side of it toward the centre of the cell. If a line through the centre of the nucleus and the centre of the cell be called the axis of the cell, these masses occurred in or near the cell axis. Occasionally faint radiations could be detected coming from the region of the central masses just mentioned and extending toward the periphery of the cell.

A more careful study of later and more favorable preparations demonstrated the common occurrence, in the cells of the brain and ventral ganglia, of such cytoplasmic structures as are represented in Figures 1–9.

Figures 1, 2, and 3 represent cells from the subœsophageal ganglion of a normal worm, — that is, one that had not undergone regeneration. These cells are from a preparation which was fixed in strong Flemming's fluid for forty-eight hours and stained in iron-hæmatoxylin. The cells are typical unipolar nerve cells, lying in the ventral anterior region of the ganglion, with their processes extending dorsally. Figure 4 represents a cell from the dorsal posterior border of the brain of the same animal.

a. The Nuclei.

The nucleus of the nerve cell has a characteristic appearance. In osmic acid preparations it is nearly always spherical, or departs only slightly from that form, as in Figures 2 and 4. In sublimate preparations, however, there are often irregularities in form, as seen in Figure 5. From a general comparison of my osmic-acid preparations with those fixed in sublimate, I am inclined to believe that the nuclei often suffer distortion in sublimate, and that the spherical form represents a more faithful fixation. The excentric nuclei are never flattened nor invaginated, except in a few cases in which I have observed that one side of a nucleus was indented in such a way as to suggest mechanical distortion due to fixation. So fluid are the contents of the nuclei that it is not strange that such distortions should sometimes occur. These indentations, which are probably artificial, had no regularity in form or in position.

The nuclei must contain a very large proportion of fluid material which is not coagulated by the fixing agent. In both stained and unstained preparations the nuclei are much clearer than the surrounding cytoplasm. They contain a very small amount of matter that takes stain.

With very rare exceptions, there is but one nucleolus. It is always excentric, often very near the nuclear membrane, and generally departs little from the spherical form. In osmic-acid preparations the nucleolus is always sharply outlined and never exhibits anything but a perfectly homogeneous structure. It takes a quality of stain different from that of the chromatin, being somewhat brownish instead of dark blue or black. This is doubtless due to the fact that it does not take or hold the stain as strongly as the chromatin, the brown color being due to the osmic acid.

The chromatic substance is scattered about in the form of small granules, approximately spherical in shape but more or less irregular. They are often not sharply outlined and appear as if imbedded in some achromatic substance. The chromatic granules are mostly collected about the periphery of the nucleus where, with the achromatic substance, they form a very loose, irregular network. Many of the chromatic masses appear to lie directly upon the nuclear membrane. The central region of the nucleus is often quite free from chromatic material. The occurrence of small sharply stained chromatic granules close about the nucleolus, or lying directly upon its surface, is a very common condition. Figures 1, 2, and 3 show these well.

Rather coarse strands of achromatic material form more or less of a network between the chromatic masses. There is often a tendency toward a radial arrangement of this network about the nucleolus, which appears supported within the nucleus by that means (Figures 3 and 4). The nuclear membrane is always very sharply outlined, being emphasized at the median plane of focussing by the occurrence of chromatic masses upon it.

b. The Cytoplasm.

Very little can be said as to the finer structure of the cell protoplasm. The most careful examination fails to reveal its precise nature. It varies in degree of homogeneity somewhat according to the size of the cell. In the smaller cells (Figures 4, 7, 8, and 9) it usually appears compact and fairly homogeneous. In larger cells (Figures 2 and 3) it is much less homogeneous, and there is a tendency toward the formation of large vacuolar spaces, as seen at the process end of the cell in Figure 2.

The substance of the fixed cytoplasm, as it appears to the eye, may be said to be of four kinds. There is (1) a perfectly homogeneous "ground," represented by the lightest areas in the figures; (2) material which gives the impression of being very finely granular; in the smaller cells this material is quite evenly distributed, while in the larger cells it tends to concentrate in regions, giving the cytoplasm a blotchy appearance; (3) rather conspicuous granules or masses staining fairly deeply and often surrounded by an area within which the material of the second class is less dense, as best seen in Figures 2 and 3; (4) fine fibres irregularly distributed throughout the cell body, but often appearing to be associated with the more conspicuous granules and sometimes occurring about granules as centres of radiation, as can perhaps be recognized in some places in Figures 2 and 3. It is possible that the granules of the third class may be due simply to concentration of the finely granular material of the second class.

To what extent these appearances in the fixed cell correspond to structure in the living cell cannot be said. The occurrence of the larger granules or masses within clearer areas suggests coagulation effects, the substance of the clearer area having been massed at the centre of it by the action of the fixing fluid. It is quite likely, however, that there may be some structural basis for this effect. The occurrence of fibres radiating from these larger granules suggests that they may mark the more important centres of a cytoplasmic network.

In some cells, especially in certain ones of the brain, a system of fibres could be detected lying at the periphery of the cell and extending out into the nerve process.

In the larger cells no definite, sharply outlined cell membrane is ever seen. The cells appear to be enclosed by fine connective-tissue fibres, which form more or less of a capsule about them. Small nuclei, doubtless of non-nervous nature, often occur about the nerve cells, as seen in the regenerated cell of Figure 11 (Plate 2).

c. *The Centred System.*

Evidences of concentric and radial structure are commonly seen, but the exact nature of this structure would be overlooked except in the most careful study, with the aid of oil immersion lenses.

Cells of the first type. — The conditions represented in Figure 1 may be considered typical for a large number of cells. This cell is from a section 6⅔ μ thick. In focussing up and down upon the section the eye is caught by a granule which is conspicuous by its size, sharpness of outline, and depth of stain. If it were a matter of a few cells or a single one, this might be a chance condition, but when a similar condition is commonly found in the observation of a large number of cells, we must conclude that we have not to do with an accidental granule. Moreover, when such a conspicuous body is found to occupy a definite and constant position in relation to other parts of the cell, and when it is found to be the centre of a system of radiating fibres, it is evident that the whole structure is one of importance. In speaking of the centre of this structure as a "conspicuous granule," it is not meant that it is the first thing that catches the eye in a casual glance at the cell. Many of the granules or masses, which have been described as forming the third class of constituents of the fixed cell, may be more prominent because of their greater size and their frequent occurrence within a clear area. But these larger masses differ from the central granule in being less deeply stained and less sharply outlined. The central granule is conspicuous as compared with any bodies in the finely granular cell substance. When once found, it is generally very easy to distinguish it from other elements contained in the cell body.

The position of the central granule is characteristic. It always lies in the part of the cell opposite the nerve process, and very nearly in the long axis of the cell. It is generally very near the nucleus, as in Figures 2 and 3; but it may be farther away, as in Figure 4, especially if much the greater bulk of the cytoplasm is on that side of the nucleus.

The central body of Figure 1 is a single minute granule, and is spherical, as nearly as could be determined for a thing so small. It lies at the centre of a very small spherical space which appears in the section as a narrow clear area about the granule. The clear area is not sharply outlined.

Several extremely delicate radiating fibres extend outward from the central granule into the surrounding cytoplasm. Those represented in the figure all lay in a plane parallel to the surface of the section. It was rarely possible to detect a fibre if it was so oblique that it did not come into the focal plane all at once. Generally not more than six or eight of these fibres could be detected. In all of the first nine figures can be seen fibres which extend well out toward the periphery of the cell. Many of the radiations, however, could not be traced so far. So fine are these radiations that it is only with the greatest difficulty that anything as to their nature can be made out. The more conspicuous radiations appeared to be made up of minute granules arranged in line. The finer radiations show no evidence of granules, appearing rather as most delicate hyaline threads. However, radiations were found exhibiting both characters at different parts of their course. It is therefore probable that these radiations consist of achromatic fibrils bearing more or fewer granules along their courses. The granules may be so thickly set that the radiation appears as a line of granules, or they may be absent, when only the achromatic fibril can be seen.

If a distinct radiation be carefully traced, it will be seen that at intervals there occur some fairly conspicuous granules, which are more easily seen because of a surrounding clear space. Figures 1, 2, and 3 show this to some extent, but the fact is much more clearly brought out in some of the regenerated cells to be described later (Plate 2, Figures 10–13).

Very often these granules, which occur along the course of a radiation, mark the point of divergence of secondary fibrils. In Figure 1, if the radiation which is most nearly in the axis of the cell be followed for one-third its length from the centre, a granule will be seen from which two fibrils proceed on toward the periphery of the cell. Figure 2 shows similar conditions, but the regenerated cells (Plate 2, Figures 10–13) exhibit this arrangement more clearly.

Figure 2 represents a cell of the same type as that of Figure 1; but the central body is much nearer the nucleus, and is either elongated or else consists of two granules very close together. The central clear space is confluent with the clear spaces around two granules that lie in the courses of radiations from the central body.

Figures 5 and 6 are from acetic-sublimate preparations stained in iron-hæmatoxylin. They represent cells very similar to those just described. The attempt has been made to show something of the difference between the appearance of an osmic-acid and a sublimate preparation. The nuclear structures are fairly well brought out by the sublimate, but not so clearly as by the osmic acid. The structure of the cytoplasm is much less definite, but as far as it could be made out, it agrees with the description given for osmic preparations. Both cells show some evidence of an arrangement in the cytoplasm concentric about the centre of the radiating system. This is especially well marked in Figure 6.

Cells of the Second Type. — Figure 3 represents a cell in which a further complication of the radial system is introduced. The central body in this case appeared very large and of irregular form, but careful focussing resolved it into three granules of different sizes. They are surrounded, not by a clear space, but by a region which appears dense and very finely granular. The limit of this region is emphasized, in the section, by the occurrence of a number of stained granules which are arranged in the arc of a circle about the central granules. The radiations from the centre can be seen to include in their courses certain conspicuous granules lying in this arc. This finely granular region is evidently comparable to the "sphere" described by many authors. Figure 3 is strikingly similar to some of the figures in Bühler's paper of 1895.

Figures 7, 8, and 9 (Plate 2) represent small cells of this type from the brain. In Figure 7 the body of the sphere differs in no visible way from the surrounding cytoplasm, but a number of granules form a complete circle about the central granule. In Figure 8 the entire radiating system is very faint, but there are evidences of two concentric circles. The structure in Figure 9 is very similar to that in Figure 3. The central body may consist of two granules, but they could not be clearly separated.

Figure 4 (Plate 1) represents a condition frequently met with. There is, in the cell drawn, a condensation of finely granular cytoplasm extending halfway from the centre to the periphery of the cell. This is probably in no way comparable to such a sphere as is shown in Figures 3 and 9. It is not sharply outlined, and it lacks the bounding layer of granules. The body at the centre of this finely granular region is surrounded by a clear space; and radiations, lacking, however, any conspicuous granules, extend toward the cell periphery. Cells of this character may best be considered as belonging to the first type (Figure 1), as far as the structure of the radiating system is concerned.

2. The Nerve Cells of Nereis.

In the ventral ganglia of Nereis are certain large cells situated near the median plane and having their nuclei in their dorsal or process ends. The nuclei are often flattened or invaginated on the side toward the cell centre. In one very large cell with an invaginated nucleus an intensely stained granule was seen lying in the concavity of the nucleus, and around the granule were several fine radiations. The cytoplasm was completely decolorized, the granule mentioned being the only stained object in it. This appeared to be a good case of the "centrosome." Nereis was not studied sufficiently to determine whether such structures are commonly present.

3. Outline of the Process of Regeneration of the Brain and Anterior Ganglia.

The regeneration of the anterior ends of the worms (Allolobophora) took place substantially in the manner described by Hescheler ('98). I have made no preparations at periods of regeneration earlier than seven days.

Figure 14 (Plate 3) represents a parasagittal section of the anterior end of a worm after seven days' regeneration. The limit of the old tissues is easily distinguishable in all such early stages, and, in fact, there is no difficulty, up to a comparatively late period, in distinguishing old tissues from those that are being regenerated. In Figure 14 the old epidermis ends at points designated by the asterisks. The new epidermis, between these points, is considerably thinner than the old. Underlying the new epidermis is a solid cicatricial mass of considerable thickness. The circular and longitudinal muscle layers are seen to end abruptly against the inner surface of the cicatricial mass. The anterior end of the alimentary canal is closed by the cicatrix and may remain closed for several weeks. The nerve cord also ends abruptly against the cicatricial mass. Even after the regenerating part of the cord is well established, the limit of the old cord is sharply indicated by the end of the old sheath, the inner homogeneous layer of which generally curls outward and backward a little at its cut end. In the preparation from which Figure 14 is taken, a more or less lens-shaped mass of newly formed cells lay near the end of the old cord. This is better seen in Figure 18 (Plate 4), which shows the end of the cord drawn to a larger scale, — 160 diameters. Two mitotic cells (Figure 18, *cl. mit.*) are seen in the lens-shaped mass. The origin and nature of them will be discussed later (page 121).

Figure 15 represents a parasagittal section through the anterior end of a worm after sixteen days' regeneration. The regenerated parts are, as in the younger stage, included between two asterisks. The regenerated epidermis remains thinner than the old epidermis. A thin layer of circular (*mu. crc.'*) and of longitudinal muscle fibres (*mu. lg.'*) has been laid down in the conical regenerated end. The end of the old nerve cord is sharply marked by the extent of the sheath (*tu.'*) and the abrupt termination of the mass of ganglion cells. Extending forward from the end of the old cord is a fibre tract, which runs out to the apex of the cone, lying ventral to the alimentary canal. The fibres become less distinct anteriorly, and are lost in a mass of cells underlying the epidermis. Along the ventral border of this fibre bundle is a thin layer of cells (*gn. nov.*) whence are to be derived the cells of the regenerated ganglia.

Figure 16 is from a parasagittal section of a worm after twenty-four days' regeneration. The regenerated parts are included between two asterisks. There is much variation as to the relative times of appearance of the regenerated organs. In Figure 15 (16 days) the regenerated muscle layers are well established. In Figure 16 (24 days) only a few longitudinal muscle fibres in the ventral part of the regenerated cone are to be seen.

In the sixteen-day preparation (Figure 15) the alimentary canal does not open to the exterior. In Figure 16 (24 days) the breaking through of the alimentary canal to the exterior is nearly, if not quite, accomplished. According to Hescheler the alimentary canal grows forward to meet a stomodæum or invagination of the epidermis, and the opening to the exterior is effected by a rupturing of the epithelial and epidermal layers, the epidermal invagination becoming the mouth cavity and the region posterior to the point of rupture becoming the pharynx. In the preparation from which Figure 16 is taken, the epidermal invagination has progressed to meet the alimentary epithelium. In some sections a delicate layer of the epithelium was found still closing the end of the canal (see Figure 16). In other sections this layer was broken away, probably accidentally.

As to the nervous system, Figure 16 represents a much more advanced stage of regeneration than Figure 15. In this advanced stage the end of the old cord is as sharply marked as ever. The new fibre tract has extended forward to encircle the alimentary canal, the two branches uniting in a small mass of cells (*gn. su'oes.*) above the stomodeal invagination. This mass of cells constitutes a well-defined brain fundament.

After the condition of Figure 16 has been reached, we have to do only with growth and segmentation of the parts already laid down. I made no preparations of material regenerated more than forty days. There is much individual variation as to the rapidity of the process. In one animal after thirty-four days' regeneration the brain was scarcely smaller than in the normal worm and had come to occupy its position in the third segment. The number of segments regenerated in this case could not be determined. The brain and anterior end of the ventral cord in this animal could hardly be distinguished from those of a normal worm except for the occurrence of numerous dividing cells at the anterior tip of the cord and in the posterior dorsal region of the brain. The sheaths enclosing the new nervous parts were well developed. But in most of the worms, after thirty-five to forty days' regeneration, the regeneration of the nervous parts had not reached so advanced a stage.

The foregoing statements are sufficient to give a general outline of the process of regeneration. Hescheler ('98) has described the process in more detail; as far as my observations go, my results agree with his. I now propose to discuss the origin of the new nervous parts more minutely.

4. Origin of the Cells of the Nervous Fundaments.

According to Hescheler the cells which give rise to the regenerated brain and ventral ganglia are derived chiefly from the epidermis. The cicatrix is at first composed of lymph cells and spindle-shaped cells of doubtful origin. Later appear cells with nuclei like those of the epidermis. These he believes to be derived chiefly from the epidermis, but some may come from the alimentary epithelium and some from certain large nuclei found in the muscle layers. In addition to these sources there is mitosis within the old nerve cord which doubtless gives rise to cells that assist in the regeneration.

From my own preparations similar conclusions as to the origin of the regenerated nervous elements must be drawn, except that only in rare cases is there any evidence that cells of the old cord assist in the regeneration.

In the preparation from which Figure 14 is taken, the cicatrix (*cic.*) is a solid mass composed mainly of greatly elongated spindle-shaped cells with small oval or elongated nuclei. Figure 17 represents a number of these cells more highly magnified. The nuclei are totally unlike epidermal nuclei, being much smaller, of different shape, and lacking a prominent nucleolus. Epidermal nuclei drawn to the same scale as Figure 17 may be seen in Figures 43–45 (Plate 6). There are also present in the cicatricial mass some very small spherical nuclei. These are doubtless nuclei of lymph cells, for they present exactly the appearance of the nuclei of the lymph cells commonly found in the body cavity. These lymph cells, in some preparations, are much more numerous in the regenerating region than elsewhere, often occurring in almost solid masses.

There is individual variation as to the character of the cicatrix. In most of my preparations of early stages (7 to 11 days) the cicatrix was composed chiefly of the spindle-shaped cells. In one eleven-day preparation, however, there was only a small mass of cells underlying the regenerated epidermis, and this mass was composed mainly of cells with large nuclei having all the characters of epidermal nuclei. These cells were actively dividing. Figure 57 (Plate 8) shows a group of these nuclei.

According to Hescheler the spindle cells give rise to the new muscle layers. In my preparations of later stages, too, these spindle cells are seen to be taking the direction of the fibres of the two muscle layers in such a way as to leave no doubt as to their fate. After the first week or two the spindle cells form a smaller proportion of the cicatricial mass at the regenerated end, this fact generally being due to the accumulation of these cells into the new muscle layers. The remaining scattered elements are either lymph cells or cells with large nuclei of the epidermal type. The latter cells tend to collect in the region into which the regenerating cord is extending.

The forward growth of the fibre bundle precedes the accumulation of cells about it ventrally and laterally in the position of the ganglionic masses.

Of the three kinds of nuclei to be met with among the incompletely differentiated cells of the regenerating region, there is no evidence that either the lymph nuclei or the nuclei of the spindle cells give rise to new nervous elements. The nuclei of these two types are totally unlike the nuclei which are first found associated with the new nervous parts, and there is no evidence of a transformation of one kind into the other. Granting this, the origin of the new nervous elements must be referred to the larger nuclei, those of the epidermal type. That these large nuclei are derived from the epidermis, there is good evidence in the preparations.

The formation of a new epidermis over the cicatrix offers some interesting problems, which, however, require a study of earlier stages of regeneration than any I have worked with. After seven days there is always found over the cut end of the worm a continuous thin layer of more or less flattened epidermal cells, and a thin layer of cuticula also is already formed. In rare cases I have found mitosis in the epidermis at this early stage. At later stages there is abundant mitosis in the new epidermis, and in one case numerous dividing cells were found among the basal or subepidermal cells back through several uninjured segments. Some signs of *amitosis* were found in the regenerated epidermis, but not conclusive evidence. Nuclei were found with two nucleoli, and several columnar epidermal cells were found containing two nuclei pressed so closely together that their contiguous surfaces were quite flat, suggesting that there had been a direct division and that the two nuclei had not yet moved apart. In one case four nuclei in a common cytoplasmic mass were found so closely pressed together that the group presented the appearance of the four-cell cleavage stage of an egg. No nuclei in process of constriction were observed.

The new epidermis having once been established, there is little room for doubt that its later increase is effected by the mitotic division of its own cells. It is after the first week that we find evidence that not all the cells produced by the epidermis are destined to remain epidermal cells.

The normal epidermis consists of a single layer of columnar cells with some basal cells of irregular form wedged in among their deep ends. The nuclei of these basal cells are unlike those of the columnar cells. They are smaller, often lack a prominent nucleolus, and generally present a darker appearance, due to a greater proportion of chromatic material. In the regenerated epidermis places are often found where certain cells, with nuclei of the kind characteristic of the columnar cells, tend to form a second layer. Single cells are found so placed as to suggest that they are being pushed down below the surface layer. Others, apparently, have been quite displaced from their superficial position and lie free underneath the epidermis. There may frequently be found small masses of cells with nuclei precisely like those of the epidermis, lying close to the deep surface of the epidermis, and so far removed from any other tissue containing similar nuclei that their epidermal origin is beyond doubt.

The surface of the regenerated end is often very uneven in the early stages. The new epidermis is thrown into folds or marked by elevations and pits. At an inner angle of a sharply invaginated region of epidermis may sometimes be seen evidences of an inward proliferation of the epidermal cells. There is also some evidence, as Hescheler finds, that not only single cells, but also considerable masses of cells, may be pushed in and separated from the surface layer. Small local invaginations are sometimes found which are nearly closed over, and in one or two places I have seen small cavities completely enclosed by epidermal cells, as if small invaginations had become closed over and had sunk beneath the surface.

In one animal of twenty-four days' regeneration the new nerve cord was already well established. The region of the epidermis nearest the anterior end of the regenerating cord consisted of loosely aggregated cells. Between this region and the end of the cord were numerous scattered cells with nuclei like those of the epidermis and also like those of the cells already definitely associated with the new cord. The scant cytoplasm of these scattered cells was more or less drawn out to spindle shape, and most of the cells were placed with their long axes extending in the direction of the nerve cord. Similar conditions were found in other animals. The appearance indicates a separation of cells from the epidermis and the migration of these cells in toward the regenerating cord.

All the facts taken together justify the belief, as Hescheler concludes, that the epidermis is an important source of cells that take part in regeneration, and especially of cells that go to the regenerating cord.

There are other possible sources of the nuclei which resemble those found in the regenerating cord. The alimentary epithelium contains such nuclei, and it is not impossible that it may furnish cells for the regeneration of other organs, but there is no good evidence for believing that it does so to an important extent in the case of the nervous system. There are scattered nuclei among the muscle fibres, and elsewhere, which resemble the epidermal nuclei, and these may also take part in regeneration.

Three facts justify the conclusion that the cells of the new brain and ganglia are mainly of epidermal origin. (1) In the early stages of regeneration the nerve cord is in more intimate relation with the epidermis than with any other tissue. (2) The new nervous parts are laid down in a region whose cells (with the exception of the lymph cells and the spindle cells) are doubtless derived mainly from the epidermis. (3) There is an apparent inward shifting of cells from the epidermis to the new cord.

One possibility remains to be considered. Hescheler finds, in regenerating worms, abundant mitosis in the ganglia of the old cord back through some fifteen segments, and concludes that the old cord furnishes some material toward regeneration. This increase of cells in the old cord has not been commonly found in my preparations. In most of the series that have been carefully studied there was no evidence whatever of mitosis or increase of cells in the old cord. In one or two animals a condition was found similar to that described by Hescheler. In one worm of ten days' regeneration were found several masses of cells presenting an appearance unlike anything to be found in a normal ganglion and containing so many mitotic cells as to indicate a rapid increase. The preparation included nine segments back of the injured segment. The most posterior mass of proliferating cells was in the eighth segment. Several similar masses occurred in ganglia of more anterior segments and occasionally isolated dividing cells were found.

In the worm from which Figure 14 (Plate 3) is taken, the mass of cells which has already been mentioned (page 116) as lying at the anterior end of the old cord is doubtless of the same nature as those found farther back in the other animal. This preparation included only five segments and no similar masses were found in other segments. In appearance the cells of this mass (Plate 4, Figure 18) are precisely like those of the more posterior groups of the other worm, and unlike any found in the near-by cicatrix. These facts, and the position of the mass well within the limit of the old cord, make it scarcely to be doubted that the mass owes its origin to the increase of cells of the old cord, rather than to any cells which may have wandered in from the cicatrix.

It is the occurrence of masses of actively dividing cells, rather than the character of the individual cells or nuclei, that distinguishes this from the condition found in the cord of a normal worm. The nuclei of these cells differ in no marked way from nuclei which may be found in the ganglia of uninjured worms. They are smaller than the nuclei of the large nerve cells, but they show the same structure as to chromatic material and the presence of a single large nucleolus. Figure 57 (Plate 8) shows some cicatricial cells of the epidermal type, but the same drawing would represent equally faithfully a group of cells from one of these masses in the old cord. The nuclei differ from any found in the normal cord mainly in being more or less elongated. In Figure 18 it can be seen that the nuclei in the thin posterior end of the mass are more elongated than those in the thicker anterior part of it. This condition suggests a mechanical deformation of the nuclei as a result of the rapidly growing mass pushing its way back into the tissue of the cord. There was scant cytoplasm about these nuclei and no definite cell outlines, as is also the case in the nuclei of Figure 57.

The absence of this cell increase in the old cord of many animals shows that it is not a necessary feature of the regenerative process. The presence of one of these masses of cells exactly at the region of injury, as in Figure 18, makes it probable that it provides material for regeneration. It can hardly be supposed that an increase of cells in ganglia situated five or ten segments back of the region of injury has anything to do with the actual regeneration of new ganglia anterior to the old cord.

5. Histology of the Nervous Fundaments.

Having considered the origin of the cells which constitute the fundaments of the brain and new ganglia, I now propose to consider certain questions pertaining to the development of the nerve cells from the cells of these fundaments. The main purpose at this point is to determine if any polar structures of a mitotic cell pass into the resting cell, persisting through all the stages of growth and differentiation to become, or give rise to, the centred system of the mature nerve cell. It is therefore necessary to examine the mitosis, and especially the later stages of it, in the cells of the fundaments, and to find, if possible, a series of cells which shall represent successive stages in the development of nerve cells.

Preparations from the later stages of regeneration were found most favorable for this purpose. In worms of from thirty to forty days' regeneration the new brain and ganglia were represented by large masses of cells, among which dividing cells were very abundant. In the same animals the differentiation of nerve cells was in progress, and frequently a single section contained not only dividing cells with scant cytoplasm, but also large, apparently fully developed nerve cells, and perhaps many intervening stages.

In the earlier stages of regeneration, cells in a solid mass, showing no signs of segmentation, are found lying laterally and ventrally about the fibre bundle which has previously marked out the position of the nerve cord. In the brain fundament a similar mass of cells lies about the neuropile, chiefly on its dorsal and posterior border, as seen in Figure 16 (Plate 3). This position of the cells is characteristic. These cells have a very small amount of cytoplasm, so that the fundaments look like masses of solidly packed nuclei. There are no distinct cell outlines, but where the nuclei are less solidly aggregated an irregular mass of cytoplasm may be found collected about each one. At sufficiently early stages the nuclei are all alike, ellipsoidal or spherical in form, and generally have a single large spherical nucleolus. Nuclei without a nucleolus are often found. They may belong to cells which have recently divided. The absence of the nucleolus is often associated with a condition of the chromatin which indicates a recent or approaching division of the nucleus.

At later stages of regeneration many of the nuclei lying deeper in the mass become larger and more nearly spherical and accumulate a considerable body of cytoplasm about them. *Cells which assume this character have ceased dividing and are in process of development into nerve cells.* The nuclei on the outer borders of the fundaments retain their embryonic character and continue actively dividing.

The more advanced the stage of regeneration, the more nearly have the deeper cells attained the character of typical nerve cells.

Figure 20 (Plate 4) represents a parasagittal section through the brain of a worm after thirty-four days' regeneration. The regenerated brain is smaller than the normal brain. (Compare Figure 20 with Figure 19, a similar section from the corresponding region of the brain of a normal animal.) The cells occupy the posterior and dorsal border of the neuropile. This relation of cells and fibre mass is found at the earliest stages in the fundament (Figure 16) and persists through all the later development. It is characteristic also of the normal brain. (Compare Figure 19.) In the deeper part of the cell mass (Figure 20) may be seen a number of pear-shaped cells with their processes directed toward the centre of the neuropile. These cells are, to all appearance, fully differentiated nerve cells, like those occupying similar positions in the normal brain. Between these cells and the periphery are smaller cells, some of them pear-shaped and with processes, others in which no processes could be found. About the extreme posterior border is a mass of nuclei with indefinitely assignable cytoplasm and exhibiting abundant mitoses, — exactly the condition which the entire cell mass of the fundament presents at earlier stages.

It is evident, then, that the deeper cells are the first to become differentiated into nerve cells, while the cells on the periphery of the mass long retain their embryonic character and continue dividing to give rise to new elements.

In the normal brain (Figure 19) the large typical nerve cells occupy a deep position. About the posterior border of the brain are smaller cells, some pear-shaped and with processes, others apparently lacking processes. There are also nuclei like those of the smaller pear-shaped cells, but having very scant cytoplasm, or there may be several of them lying near together in what is, to all appearance, a common cytoplasmic mass. Such nuclei, or groups of nuclei, differ in no visible way from the actively dividing nuclei which constitute the early fundaments. They resemble likewise the peripheral layer of nuclei present at so late a stage of regeneration as is seen in Figure 20. If it could be assumed that the embryonic development of the normal brain is similar to the regenerative development, there would be little reason for doubting that the small cells and "indifferent" nuclei of the posterior border of the normal brain represent the mass of embryonic cells which have given rise to the differentiated nerve cells. There is little or no reason for regarding them as neuroglia cells. The nuclei which, beyond question, belong to the neuroglia are of an entirely different character.

Conditions in the regenerating cord are similar to those in the brain. The deeper cells are first differentiated into nerve cells, while the cells along the ventral border and at the anterior tip of the ganglionic mass continue dividing long after the deeper cells have attained the size and form of typical nerve cells.

6. MITOSIS IN THE NERVOUS FUNDAMENTS.

Figures 21 to 30 are taken from the actively dividing cell masses in the regenerating brain and cord of animals after about five weeks' regeneration. As already mentioned, these cells have very little cytoplasm and there are no definite cell outlines. Often the nuclei are packed so closely together as to appear imbedded in a common cytoplasmic mass. In the resting cells of this character I have been unable to discover any structure which could, beyond doubt, be taken for a centrosome. If present, it must be an extremely minute body, and all the conditions are most unfavorable for its discovery. It is not until the cell has passed into the earlier stages of mitosis that an unquestionable centrosome is to be observed.

Cells in the early prophase are numerous and generally present the appearance of the cell at the right in Figure 21. Such cells are conspicuous objects because of their sharply defined spherical outlines and the clear area surrounding the chromatic elements. The sharp outline is due to the presence of a distinct membrane, and, for reasons to be given later, this membrane must be considered to be a cell membrane and not an expanded nuclear membrane. At some time during the formation of the chromosomes the nuclear membrane disappears.

The appearance of the cells at this stage indicates a condition of turgescence. The entire cell contents, cytoplasmic as well as nuclear, with the exception of the chromatin, are in a highly fluid condition, judging by the absence of stained substance (except the chromatin) in the preparations. That there is a swelling of the cell during the beginning of mitosis is proved by such conditions as are seen in Figure 21. Here are two dividing cells close together. The one at the right is in the prophase, while the one at the left is in the metaphase. (The axis of the spindle in the latter cell is oblique to the plane of the section, and one pole has been cut away. The remainder of the cell was easily identified in the next section.) The two mitotic cells have increased in volume; and the large nucleus of another cell, caught between them, has been pressed out of shape by the combined pressures from the two swelling cells.

The smallness of these cells and the large number of chromatic elements make it difficult to determine the exact manner of formation of the chromosomes. One or two nuclei have been found which gave some evidence of a spireme condition of the chromatin, but, aside from these, the earliest condition of the chromosomes was that seen in Figure 21, where the chromatin is in the form of large, nearly spherical granules. The number of chromosomes could not be accurately determined.

The presence of a centrosome was not observed until the spindle had been well formed. The two granules lying at the edge of the chromatic mass in Figure 21 (cell at the right) may be the centrosomes which have not yet taken their position at opposite poles. Cells were found where the two poles of the spindle had been established, and the chromosomes were not yet aggregated into an equatorial plate. Sometimes an extremely minute centrosome could be distinguished at the poles of the spindle at this stage. When the cell is in the metaphase the centrosomes are generally conspicuous objects, although there is much variation as to their size. The half spindle in Figure 21 (cell at the left) shows a centrosome. In Figure 22 the centrosomes are unusually large for cells at that stage. The average size of the centrosomes is perhaps fairly indicated by Figure 24.

In the metaphase the cell membrane is still present. The loss of a perfectly spherical form may mean a decreased internal pressure. Particular attention is directed to the fact that there is very little stained material outside the limits of the spindle. Figure 23 represents a very typical metaphase. Except for a slight cloudiness near the poles, the cell body is quite clear. The spindle is very sharply outlined, and at its poles are minute deeply stained centrosomes. No polar radiations are visible. In Figure 22, except for a few indefinite irregular masses of unstained material, the body of the cell is clear. In this case, however, a few very indefinite polar rays could be seen. In Figure 24 there is an unusually large amount of material, apparently finely granular, in the region of the two poles, and into this material extend some well defined polar radiations. The half spindle of Figure 21 shows an extreme case of the presence of solid substance outside the spindle.

In the metaphase, then, the body of the cell is generally clear. If solid material be present outside the limits of the spindle, it tends to be accumulated about the poles and may be associated with polar radiations.

The division of the chromosomes must take place at a very early stage of the equatorial plate, for in most of the cells in the metaphase the chromosomes are in a double layer.

The region occupied by the spindle always presents a compact and homogeneous appearance, although not deeply stained. A limited number of conspicuous fibres may be seen lying upon the surface of the spindle. These are quite likely "mantle fibres" attached to the chromosomes. They exhibited a finely granular structure.

The anaphase must be a period of very short duration, for figures in which the chromosomes were just separating, or had traversed less than half the distance toward the poles, were rarely found. The few that were found exhibited no unusual features.

Figure 25 represents a cell in which the chromosomes have nearly completed their migration toward the poles. At the right pole the chromosomes are aggregated into a nearly solid mass. At the left pole they are still somewhat scattered. The sharp cell outline is still preserved, indicating the presence of the cell membrane. There is a very slight equatorial constriction of the cell. The body of the cell, outside the spindle figure, is perfectly clear except for a slight trace of indefinite material on one side of the spindle. The interzonal filaments occupy a barrel-shaped region. This shape of the figure is characteristic for this phase, and cells in this condition were very abundant. The barrel-shaped figure lies sharply outlined in the surrounding clear space. The interzonal filaments are of granular appearance, and some irregular dark masses occur upon them.

The presence of a centrosome at the left pole of the figure is doubtful. The black granule which appears to occupy the position of the centrosome is much larger than the centrosome usually is at this stage. It is possibly an aberrant chromosome. The axis of the figure is oblique to the plane of the section, the left end being higher. This position favors the obscuring of a centrosome. Although the chromosomes at the right pole are in a nearly compact mass, something of the form of individual chromosomes is still to be distinguished. The centrosome is unmistakable, lying at the apex of the old spindle. The chromatic mass is more smoothly outlined on the polar side, being concave toward the centrosome. This condition is characteristic and is seen to better advantage in the cell represented in the next figure.

Figure 26 is a somewhat later stage than Figure 25, as is shown by the complete consolidation of the chromosomes and the deeper equatorial constriction of the cell. The cell membrane is still distinctly present, although at some regions it appears fainter and less clearly defined. The cell body, outside the region of the spindle, is still nearly clear. The figure itself is barrel-shaped, as before, but the interzonal filaments are more sharply bent at the equator. Each group of daughter chromosomes appears to have fused to a solid mass. In the mass at the right pole the position of a lagging chromosome is indicated by a chromatic process extending toward the equator.

The important feature at this stage, in its relation to later stages, is the condition on the polar side of the chromatic mass. Each chromatic mass is concave on the side toward the centrosome and convex on the opposite side. The polar and equatorial surfaces of the chromatin lie in the surfaces of two spheres whose common centre is marked by the centrosome. These conditions point to the centrosome as the centre of the forces by which the chromosomes have been moved toward and grouped around the poles.

Between the centrosome and the polar surface of the chromatin is a region otherwise bounded by a conical surface extending from the centrosome, as apex, to the nearer outer edge of the chromatic mass. This is the region originally occupied by the end of the spindle, but at this stage it is impossible to detect any fibres extending from the centrosome toward the chromatin. This polar region is stained sufficiently to be sharply outlined against the outer clear space of the cell, and yet it is so much lighter than the chromatin as to be clearly distinguished from the chromatic mass. It appears perfectly homogeneous, exhibiting neither fibres nor granules.

This condition of chromatin and polar structures persists for some time while certain equatorial changes occur. These changes include the formation of a membrane between the daughter cells (Plate 5, Figure 27). The figure meanwhile loses its barrel shape, assuming the form of two cones with apices together at the plane of the new membrane. The axis of the entire figure may become bent at the meeting of the two cones. The axis of the left daughter cell in Figure 27 is almost parallel to the plane of the section. The chromatic mass is of characteristic appearance, but the centrosome was not clearly to be made out. The axis of the right daughter cell extends obliquely upward, so that the centrosome is obscured, and the observer looks down into the concavity of the chromatic mass. The interzonal filaments are not sharply constricted at the equator. The fact that varying degrees of equatorial constriction of these filaments may be found, supports the view that the fibres are carried inward toward the axis of the figure by an ingrowing membrane. However, in most cells at this stage may be found traces of fibres still occupying the region formerly filled by the barrel-shaped figure. Such fibres are to be seen in Figures 27–29.

Two facts of importance are to be noted at the stage of Figure 27. The original cell membrane is in process of degeneration, the clear space about the mitotic figure being no longer sharply outlined. It is of irregular form, as if being encroached upon by the cytoplasm of near-by cells. The second fact is the presence of considerable loosely aggregated, rather granular material outside the limits of the constricted figure. As the volume of the constricted figure is much less than that of the barrel-shaped figure which precedes it, and as the material in question is mainly in the region formerly occupied by the barrel-shaped figure, it is reasonable to conclude that this material is some of that originally contained within the limits of the larger figure.

In Figure 28 one chromatic mass has been cut away. The centrosome of the other pole is not visible, probably because of the obliquity of the axis. Something of the lightly stained polar region can be seen. The old cell membrane has degenerated to a less extent than in Figure 27. A distinct equatorial membrane is present, and the interzonal filaments are sharply constricted. Outside the constricted figure is some loose material, as in Figure 27, and a few fibres from the chromatic mass mark the outlines of the former barrel-shaped figure.

Figure 29 is a reconstruction. The left chromatic mass was cut away from the rest of the figure, being found in an adjacent section. The old cell membrane is indistinct. The equatorial membrane is clearly present. The interzonal filaments are sharply constricted, and there is a suggestion of a "Zwischenkörper." There are some traces of fibres outside the constricted figure, but, except for these, *the space outside the figure is almost clear*. The chromatin appears enclosed in a membrane, and the daughter chromatic masses show considerable increase in size. The chromatin is in the form of large granules. At the polar side of the left chromatic mass (nucleus?), the lightly stained region and the centrosome are to be seen. These could not be seen in the other daughter cell, perhaps because of the position of the axis. The condition of the chromatin in this case is much more advanced than ordinarily when the interzonal fibres are still present.

In Figure 30 the chromatic masses are in much the same condition as in Figure 27. Owing to the obliquity of the axis of the right daughter cell, the centrosome is not to be seen. The old cell membrane is fairly distinct in some regions, but quite lacking in others. The interzonal filaments are sharply constricted. There is, again, a suggestion of a Zwischenkörper, although the concentration of the fibres may account for the darkness at their equatorial region. The important points to be noticed are, first, that the daughter-cell bodies outside the limits of the constricted figure are practically clear, and, secondly, that there is no trace of an equatorial membrane. The constriction of the old cell outlines and the constriction of the interzonal filaments would indicate that the equatorial membrane had been formed and has disappeared.

7. Development of the Nerve Cells.

The significance of these facts concerning the cell membranes will best be understood after a description of Figures 31–36 (Plate 5), which represent further stages in the progress of recently divided cells toward a resting condition. That such objects as are seen in these figures are what I have taken them to be admits, I believe, of no doubt. They occur with about the same frequency as the later stages of mitosis. Moreover, there are no other objects present which could possibly be taken for recently divided cells. They are found in pairs oftener than would occur by chance. That they seem not always to occur in pairs may be explained, in part, at least, by two facts. It is frequently observed that two sister nuclei may progress toward the resting condition with very unequal degrees of rapidity. There is much evidence that this is so in the case of these cells. Figures 32 *a* and 32 *b* represent two objects which lay so close together as to leave no doubt as to their being sister cells, but there is a marked difference in the size and compactness of the chromatic masses. It must be that one of two sister cells may so far outstrip the other in regaining the resting condition as to leave the slower cell apparently without a mate. Secondly, when two sister cells lie in different sections, having found one, it is difficult to identify the other, especially if they are not alike in appearance.

It often happens that two very similar young nuclei are found, not so very far apart, but yet farther apart than they could have been at the end of mitosis. In some regions of the fundaments of the nervous parts the cells are very loosely aggregated, the spaces doubtless being filled with fluid in the living animal. It seems probable that there may be a mechanical shifting of the less securely supported cells, merely as a result of the muscular activities of the animal. In this way young sister cells may become separated from each other.

Figure 31 represents a young cell from the ventral posterior border of the regenerating brain, together with three nuclei of adjacent resting cells. A similar object was found in the next section, but at a distance considerably greater than could have intervened between the two at the end of a mitosis. If the darkly stained object in Figure 31 be compared with the chromatic masses and their accompanying polar structures in Figures 26–30, it will readily be seen that the disappearance in the latter of that part of the spindle which lies between the two chromatic masses, and the assuming of a more nearly spherical form by the chromatic masses would result in two objects like the one seen in Figure 31. Figures 32 *a* and 32 *b* represent two young sister cells with several of the surrounding nuclei. They lay in two adjacent sections, and by means of camera drawings their centres were found to be exactly superposable.

The interpretation of Figure 31 is obvious. The heavily stained compact mass, concave upon one side, otherwise spherical, is one of two daughter chromatic masses resulting from the fusion of a group of daughter chromosomes. On the concave side of the chromatin is an area distinguished by a stain distinctly lighter than that of the chromatin, but yet sufficiently heavy to outline it sharply against the outer clear space. On the mid-border of this lightly stained region is a dark granule. This granule is the centrosome, and the lightly stained region corresponds to the polar region of the old spindle. The chromatic mass and its accompanying polar structures lie in an irregular, indefinitely outlined, clear space. This clear space is the region formerly limited by the old cell membrane, which has completely disappeared. In the later stages of mitosis represented in Figures 27–30 various stages in the degeneration of the cell membrane of the mother-cell are to be seen. The dark masses (Figure 31) lying in the clear space, on the apolar side of the chromatic mass, may be remnants of the old spindle.

In Figure 32 *a* is a similar condition of the chromatic mass with the lightly stained polar region on its concave side. No distinct centrosome was visible in this case. The surrounding clear space marks the region formerly bounded by the daughter-cell membrane. In Figure 32 *b* a more advanced condition of the chromatin is seen. The volume of the chromatic mass has increased, and this increase is attended by the separation of the chromatin into a number of large granules, between which the mass has a lighter appearance. The concavity on the polar side of the chromatin is still marked. The lightly stained polar region is sharply outlined. It has increased in volume and lost its conical form. The centrosome no longer lies at its border, but well in toward the concavity of the chromatic mass. There are some faint lines extending from the centrosome to the periphery of the lightly stained region. Chromatic mass and polar structure lie in a clear space.

Figure 33 shows two pairs of sister cells. *α* and *β* are to be regarded as the chromatic masses of one pair. The chromatic masses of the other pair lie one over the other. *γ* is the upper of the two masses, and the outline of the lower is indicated by *δ*. In *α* and *β* the condition of the chromatin is much as in Figure 32 *b*. *α* shows the concavity of the chromatic mass, the lightly stained polar region, and the centrosome at its apex. β is in an unfavorable position for the observation of the polar structures. α and β lie in a common clear space, which shows a constriction in the region between the two chromatic masses. This common clear space is that formerly bounded by the mother-cell membrane. The equatorial membrane, separating the two daughter cells, has disappeared. γ and δ are unfavorably situated for study.

Figures 34 and 35 represent two young cells whose sister cells could not be identified. The chromatic masses are in the same condition, both showing the polar concavity and the loosening of the chromatin into large indistinctly separable granules. In both cases the lightly stained polar region and its centrosome are present, and in both cases a thin layer of lightly stained material extends from the polar region along one side of the chromatic mass to its opposite end. In Figure 35 a slightly clearer area bounded by a darker ring surrounds the centrosome. In both figures a clear space surrounds the ellipsoidal body, which is composed of the chromatic mass plus its polar structures.

Figure 36 shows a group of cells from the posterior ventral border of a regenerating brain. At the centre of the group is a young cell. The chromatic mass is composed of indistinctly separated granules. On one side of it is the lightly stained polar region within which lies a dark granule, doubtless the centrosome. On the border of the lightly stained region are two dark places, but focussing shows that they are not distinct granules. There is no marked polar concavity of the chromatic mass. The surrounding clear space belonging to the daughter cell is well defined.

Above the dividing cell in Figure 29 is a young cell which shows the swelling chromatic mass, the polar region, centrosome, and the clear space about the entire stained object.

Figures 37 and (Plate 6) 38 show later stages in the condition of the chromatic masses; it is not until such stages as these are reached that answers can be confidently given to the questions, which already must have suggested themselves. How much is nucleus and where is the cytoplasm in Figures 31–36? Unless one is prejudiced by holding the belief that the centrosome must lie outside the nucleus, there are two possible interpretations of the object shown in Figure 32 *b*. It may be that the nucleus is identical with the chromatic mass, or it may be that the new nuclear membrane is represented by the elliptical outline which encloses the chromatic mass together with the lighter region at its pole. Upon careless observation with a dry objective, one would hardly hesitate in saying that the elliptical outline is that of the nuclear membrane.

According to this interpretation, the centrosome would lie within the nuclear membrane.

When the condition of Figures 37 and 38 is reached, it becomes evident that *the lightly stained polar region is not included within the nuclear membrane.*

Figure 38 shows a group of cells from the anterior end of a regenerating cord. At the centre of the group is a large nucleus slightly irregular in form. It contains a single nucleolus and is denser in chromatin than most resting nuclei. On one side of the nucleus is a lightly stained region, appearing crescent-shaped in the section, and sharply outlined against the clear space which surrounds it and the nucleus. At the mid-periphery of the light region is a dark granule. A comparison of Figure 38 with Figures 31–36 leads to the conclusion that *the chromatic masses of Figures* 31–36 *are the daughter nuclei, and the lightly stained polar regions including the centrosome are not contained within the nuclear membrane, but represent cytoplasmic structures.*

Figure 37 shows a cell from a regenerating brain. The nucleus, containing a large proportion of chromatin, still exhibits the polar concavity seen in the earlier "chromatic masses." No nucleolus is yet visible. In this case the lightly stained region on the concave side of the nucleus is of much greater volume in proportion to the nucleus than in Figure 38. Near the mid-border of it is a small deeply stained granule surrounded by a slightly clearer space. Some weak radiations proceed from the granule. In Figure 37, then, we have a nucleus, upon the concave or polar side of which is a considerable mass of cytoplasm containing a centrosome and radiations. But what of the clear space surrounding the nucleus and its polar mass of stained cytoplasm? In all the stages represented in Figures 31–38, this clear space is present. It can be interpreted only as the space originally bounded by the mother-cell membrane, which has now degenerated. In such a case as Figure 37, is the clear space to be regarded as a part of the cell territory belonging to the nucleus which lies within it, or is the entire daughter cell at this stage to be considered as included within the outline which encloses the nucleus and the dense cytoplasmic mass lying on its concave side? I hold the latter to be the correct interpretation. In all of Figures 31–38, *the lightly stained polar region containing the centrosome constitutes the fundament of the cytoplasm.* This cytoplasmic fundament increases in volume along with the nucleus, occupying more and more of the space originally bounded by the old cell membrane.

At all stages it must be said that all the *living parts,* at least, of the daughter cell are included within the limits of the nucleus and its polar cytoplasmic fundament. It may be that the surrounding clear space contains fluid material derived from the degenerated parts of the mother cell, and that the growing cytoplasm of the daughter cell utilizes some of this material. In that sense only can the clear space be regarded as actually a part of the young cell.

Figure 39 shows two cells from a regenerating subœsophageal ganglion. The nucleus at the left is in a typical resting condition, having a chromatic network and large nucleolus. Its cytoplasm is massed at one end of it and contains the centrosome surrounded by a dark ring. The nucleus at the right shows no nucleolus. The cytoplasm is massed at one end of the nucleus and contains a clear space at the centre of which is a dark granule with some weak radiations. The young cells of Figures 34 and 35 may readily be imagined to become such cells as are seen in Figure 39.

Figures 40 and 41 represent cells from the brain after five weeks' regeneration. The nuclei have the form and structure typical for those of nerve cells. The cytoplasm in both cases is collected upon one side of the nucleus and contains an unmistakable centrosome and radiations, constituting a centred system like that which has been described in nerve cells of the normal worm. No nerve processes from these cells could be seen. Cells like these may be derived from cells like those in Figures 37 and 39 simply by the increase in volume of nucleus and cytoplasm. By this growth the space originally bounded by the old cell membrane becomes entirely filled, and there is no longer a clear space about the growing cell.

In Figure 36 the large nucleus at the extreme left possesses a considerable cytoplasmic mass, which contains evidence of a centred system, although no distinct centrosome could be seen. The nucleus is peculiar in lacking a nucleolus. The cell at the extreme right has a pear-shaped form, and there is some evidence of a process at the smaller end. On the side of the nucleus toward the larger end of the cell is a region of denser cytoplasm with a lighter centre, but no centrosome or radiations can be seen.

Figure 42 shows a group of three cells from a subœsophageal ganglion of thirty-four days' regeneration. The three nuclei lie at different levels, the smallest one being lowest. A centrosome and radiations may be seen close to each of the two larger nuclei, and on the side toward the greatest cytoplasmic mass.

8. The Centrosome in the Mature Regenerated Nerve Cells.

Evidences of the presence of the centred system are to be found in cells of all sizes from those represented in Figures 40–42 (Plate 6) to the fully differentiated nerve cells such as are shown in Figures 10–13 (Plate 2), which are from a brain of thirty-four days' regeneration. The cells shown in Figures 10–12 lay side by side in a single section of the brain. Figure 10 represents a cell whose centred system places it among cells of the "first type," as described (pages 112–114) for cells of the normal animal. The whole structure in this case was unusually distinct. The centrosome (for so it can now be called) lies in the axis of the cell and so close to the nucleus that the small clear space about the centrosome is tangent to the nuclear membrane. Four distinct radiations extend nearly or quite to the periphery of the cell. They appear finely granular. The presence upon the radiations of conspicuous granules surrounded by small clear spaces can be seen. The two radiations nearest the axis of the cell appear connected by a cross fibre, which extends between two of the larger granules mentioned.

In Figure 11 there is a distinct centred system with its centrosome in the axis of the cell and near the nucleus. About the centrosome is an imperfectly described circle of granules, four of which lie on the paths of radiations from the centre. In this respect, the system is one of the "second type" (page 114), but the region bounded by the circle of granules is rather lighter than the surrounding cytoplasm, instead of denser, as in Figure 3. A small neuroglia nucleus lies close upon one side of the cell.

Figure 12 shows a clear circle tangent to the nucleus and in the cell axis. At the centre of the clear circle are three indistinctly defined granules, from which several faint radiations extend into the cytoplasm.

Figure 13 exhibits a remarkable complexity of the centred system. The cytoplasm in this case was practically unstained, yet its fibres stood out with much clearness. This cell shows to advantage the presence of secondary centres. The primary centre, or centrosome, is easily distinguished as lying in the cell axis and in being the point from which proceed several radiations which extend in straight lines almost to the periphery of the cell. Upon these radiations are conspicuous granules from which extend secondary radiations. The secondary radiations sometimes appear to connect certain larger granules on two different primary radiations. This cell is one of the first type, lacking anything that could be called a "sphere."

V. General Conclusions.

1. Mitosis in the Nervous Fundaments.

To summarize briefly the process of mitosis as seen in the cells of the ganglionic fundaments:

The prophase is accompanied by a turgescence of the cell; a distinct cell membrane is formed; the nuclear membrane disappears; the chromatin takes the form of nearly spherical chromosomes; the region between the chromosomes and the cell membrane is nearly or quite clear; a spindle appears with its centrosomes very minute, if visible at all.

In the metaphase a distinct cell membrane is still present; the spindle with its equatorial plate of chromosomes lies sharply outlined in the cell body, which is otherwise nearly or quite free from any solid material; generally the centrosomes are clearly to be seen at the spindle poles and often they become fairly conspicuous objects.

In the later phases the chromosomes separate and move toward the poles, each daughter group fusing into a compact mass, which is concave on its polar side; the centrosome remains visible at the apex of the old spindle, the end of which forms a lightly staining region between the centrosome and the concave surface of the chromatic mass; the interzonal filaments occupy a barrel-shaped region; an equatorial constriction of the cell membrane appears. The ingrowth or differentiation of an equatorial membrane between the two daughter cells follows, accompanied by the constriction of the barrel-shaped figure to the form of two cones with apices together at the plane of the equatorial membrane. As a result of this decrease in the volume of the figure, some of the substance of the barrel-shaped figure, including a few of its filaments, is left outside the limits of the constricted figure. This substance disappears later.

During the forming of the equatorial membrane, the old cell membrane (now become the daughter-cell membranes) undergoes degeneration, and finally the new equatorial membrane also disappears. At this period, then, the constricted figure lies sharply outlined in an irregular clear space which is not definitely outlined, not enclosed in a membrane, and not divided by an equatorial membrane (Fig. 30). The interzonal filaments degenerate, leaving the two daughter chromatic masses and their accompanying polar structures lying in a common clear space (Fig. 33, α and β). The chromatic mass is the nucleus; the lightly stained conical polar region with the centrosome at its apex is the fundament of the cytoplasm.

The chromatic mass, enclosed within its new nuclear membrane, breaks up into granules, which become the chromatic parts of the resting nuclear network. The polar concavity persists during the earlier growth of the nucleus.

The polar cytoplasmic fundament increases in volume along with the nucleus, the centrosome persisting through all the growth of the cytoplasm, and becoming the centre of the radiating system of the resting cell.

That the spherical membrane seen in the prophase (Fig. 21) is a cell membrane and not an expanded nuclear membrane admits of no doubt when its fate is considered. It is identical with the membrane which encloses the clear space surrounding the figure in the metaphase (Figs. 22–24), and it is this membrane which constricts during the later phases, in connection with the formation of the equatorial membrane. Moreover, the centrosome in the daughter cell lies outside the nucleus. During the prophase, therefore, we should not expect to find it within the nuclear membrane.

The peculiar feature in the reconstitution of the daughter cells is the complete degeneration, during the telophase, of all parts of the mother cell outside the limits of the spindle figure, together with the newly formed equatorial membrane. Still later, the interzonal filaments having disappeared, there comes a period when all the living parts of each daughter cell consist of the compact chromatic mass (nucleus) and its polar region (cytoplasmic fundament), including the centrosome.

2. Development of the Nerve Cells.

In the progress of the nucleus toward the resting condition there is little, if any, increase in the volume of the chromatin. The growth of the nucleus is due to the increase in its fluid contents. As nearly as can be judged, the total volume of the scant chromatin in the large resting nucleus is about the same as the volume of the compact chromatic mass immediately after mitosis.

The growth of the cytoplasmic fundament involves the assimilation of outside material and its transformation into a dense protoplasm. As already stated, the cytoplasm in the smaller cells is denser, more finely granular and homogeneous than in the larger cells. It seems highly probable that the fluid material resulting from the degeneration of parts of the mother cell is utilized by the growing cytoplasm of the daughter cells, which finally come to occupy the territory originally filled by the mother cell. It is commonly the case that the cytoplasm becomes more or less fluid during the earlier phases of mitosis, having a clear appearance in preparations; especially is this the case immediately about the nucleus or the spindle. But in the usual forms of mitosis, the cytoplasmic structure reappears throughout the body of each daughter cell during the later phases, and the daughter nucleus, during its reconstitution, lies in a cytoplasmic mass which is obviously half that of the mother cell. The mother cytoplasm undergoes only a temporary alteration in structure. In the mitosis of the cells of the ganglionic fundaments with which we are dealing, there is, on the contrary, nothing which suggests the reappearance of the old cytoplasmic structure throughout the bodies of the daughter cells, but a growth of new cytoplasm takes place progressively outward from the polar region of the nucleus. *The end of the old spindle lying between the centrosome and the daughter chromatic mass (nucleus), together with the centrosome, contains the substance which effects the regeneration of the cytoplasm.*

This regeneration of the cytoplasm of newly divided cells takes place only among the cells of the nervous fundaments. It is found in none of the other regenerating tissues. In all of the preparations from which my figures have been taken, mitosis of the ordinary type may be found in other regenerating tissues. In the dividing cells of the cicatrix there is at all phases a more or less dense mass of cytoplasm about the spindle figure. The mother cytoplasm in these cases is divided in the usual way, one half persisting, without degeneration, about each daughter nucleus. In cells of the epidermis, of the alimentary epithelium, and in nuclei lying in the muscle layers, the mitosis is accompanied by no sign of degeneration and subsequent regeneration of the cytoplasm. In cells of the brain sheath itself, or in cells lying immediately outside it, the telophase shows some dense cytoplasm about each daughter nucleus, whereas, in the same preparations, telophases within the brain fundament exhibit the conditions which have been described, — the absence of structure outside the spindle figure itself. Moreover, in the nervous fundaments, at least in their later stages, all of the mitoses are of the type described. No dividing cells whatever were found presenting conditions other than these. At what period of regeneration mitosis of this type appears, I am unable to say. My preparations have not as yet yielded stages favorable for determining conditions in the very earliest fundaments. In some preparations otherwise favorable, mitosis in the fundaments is rare or absent, indicating that the animal was killed during an interval between periods of cell increase. All of the mitoses described are from preparations in which the fundaments are well established, containing, in their deeper parts, cells which have ceased dividing and assumed the appearance characteristic of nerve cells.

It is not certain that all the products of division, in fundaments at this stage, immediately acquire a large cytoplasmic mass and become differentiated into nerve cells. It is evident that, at some earlier period of the fundament, this could not possibly be so, else the supply of undifferentiated cells would be exhausted before the needful number of nerve cells had been produced. It must be that up to a certain period one or both of two sister nuclei acquire only scant cytoplasm after a division, retaining their embryonic character for the purpose of further division. After a sufficient number of nuclei have been produced, it may be that both of two sister nuclei give rise to nerve cells, while some of the embryonic cells cease dividing without undergoing differentiation into nerve cells, remaining as the small embryonic cells seen about the posterior border of the full-grown brain. Or it may be that one of two sister nuclei gives rise to a nerve cell, the other ceasing to divide and remaining as an undifferentiated cell.

Little can be said as to the origin of the neuroglia of the regenerated brain and cord. As the new nerve cells become differentiated, there also appear among them small nuclei similar in character to nuclei — doubtless of non-nervous nature — found in the normal brain and cord. It is not improbable that cells of the early fundaments give rise to the neuroglia as well as to the nervous nuclei, — a differentiation from the indifferent cicatricial cells in two directions. It is also possible, however, that the neuroglia may be derived from cells having a common origin with those that give rise to the sheath.

3. PERSISTENCE OF THE CENTROSOME.

A definite centrosome is first to be seen at the time of the formation of the spindle in the prophase. It is an extremely minute body at that time, but during the later phases it increases in size, appearing as a single, very dark, spherical granule lying at the pole of the spindle. At the close of mitosis it increases considerably in size, and sometimes appears less intensely stained. In Figures 31–35 (Plate 5) the centrosome is seen to be much larger than at any stage of the mitotic figure. In Figure 34 it is not a spherical granule, but is somewhat elongated. In cells showing a greater development of the cytoplasm (Figures 36–38) there is generally present a large dark granule near the centre of the cytoplasmic mass. In as early conditions as those seen in Figures 32 *b*, 35, 37, and 38, the differentiation of a clear space, or a darker circle, about the centrosome, or some evidence of radiations can sometimes be seen. The centrosome is largest immediately after mitosis. As the cytoplasm increases in volume the centrosome becomes somewhat smaller. In cells like those of Figure 39, where the nucleus is in typical resting condition, the small cytoplasmic mass contains the centrosome, frequently with evidences of concentric and radiating structures about it. With further increase in the size of the cell and the volume of the cytoplasm (Figures 40–42) there is to be seen a centred system — centrosome, clear space and radiations — comparable in all respects to the system seen in the mature nerve cell.

In all stages of development between the last mitosis and the mature cell, the centrosome and its accompanying structures are so frequently met with that one is warranted in concluding that they are generally present.

It is evident, then, that the centrosome of mitosis is present during the earliest stages in the reconstitution of the daughter cells, that it persists during the growth of the cell, becoming associated with certain concentric structures and radiations, and becomes finally the central body of the complex system of radiating and intersecting fibres found in the fully differentiated nerve cell. This is true for the regenerated cells. That it will be found true in the case of nerve cells developed by the normal embryonic process is hardly to be doubted.

4. The Centrosome in Nerve Cells.

In transverse sections of a ganglion the cells are most likely to lie with their axes nearly parallel to the plane of cutting. In such sections evidences of the centred system are often to be found in the majority of the pear-shaped nerve cells. Thus the three cells of Figures 9–11 (Plate 2), from a regenerated brain, lay side by side in the same section.

A centred system is not to be made out, however, in every cell of a section. Even if the structure is one generally present, we should not expect to be able to see it in all cells, for only the most favorable conditions could bring it to view. If the axis of the cell is not approximately parallel to the plane of cutting, the cell is likely to be cut so that the centrosome is not in the same section with the nucleus, — a condition which increases the difficulty of identification. So delicate a structure may often be obscured by some of the darkly staining masses of the cytoplasm. Considering the difficulties of observation, then, the centred system can be made out in so large a proportion of cells as to justify the belief that it is a structure usually, if not always, present in the mature nerve cells.

The largest cells of the brain and subœsophageal ganglion were least satisfactory for showing the presence of this structure. It was found with greatest frequency in cells of medium size, like those shown in Figures 1–4.

The "centrosomes" and "spheres" described for nerve cells by various authors present such widely different conditions as to suggest that they are not all homologous structures. For example, the condition in the centred system as I have found it in the earthworm is in no way similar to the sharply outlined homogeneous sphere and its numerous central granules described by von Lenhossék for spinal ganglion cells of the frog. The structure in such a cell as is represented in my Figure 3 may suggest his "sphere," yet it differs in being bounded by a layer of granules which occur on the radial fibres. Von Lenhossék found no granules bounding his sphere, nor any radiations.

The structure described by Dehler resembles that of von Lenhossék, being equally unlike anything found in the earthworm.

It is not impossible that the centrosome of mitosis, persisting in the resting cell, may give rise to structures differing as widely as the centrosome and sphere of von Lenhossék and Dehler differ from the centred system which I have described. A fuller knowledge of the structures described by them is needful. As long as they can be interpreted as sections of a "spiral figure' (Holmgren), or as wandering nucleoli (Rohde), a detailed comparison of these with the centred system is hardly warranted.

The methods used by Schaffer were not specially adapted to the study of finer cytoplasmic structure. McClure examined Lumbricus and found no evidence of the centrosome. The centrosome and sphere described by him for the nerve cells of other invertebrates exhibited no radial structure comparable to the radiating system I have described. Hunter sometimes found in ascidians "well developed astral rays" proceeding from the central homogeneous sphere with its centrosomes. Such a cell as that shown in my Figure 3 presents somewhat similar conditions.

Miss Lewis found in Clymene producta a centred system, — central granules and fine radiations, — with the additional complication of a sphere having a diameter perhaps one-third that of the cell. The condensed central region of the cell in my Figure 4 resembles such a sphere.

The structures most nearly resembling the centred system seen in the earthworm are those described by Bühler ('95) for brain cells of the lizard and ('98) for nerve cells of amphibians and mammals. In the later paper Bühler finds in some cells a centred system with its one or two central granules lying close to the nucleus and fine radiations extending toward the cell periphery, and in the same cell he also finds a concentric arrangement about the centre of the cytoplasmic mass, strongly suggesting the sphere of von Lenhossék. In other cells he finds a "spiral figure" similar to that described by Holmgren. These conditions suggest that the spheres of von Lenhossék and Dehler may be structures in no way connected with a centred system of fibrils, and that both structures may be present in the same cells.

The theory of the structure of the centred system, as proposed by Bühler, is strongly supported by my results. Bühler believes that the centrosome is the insertion point of the stronger fibrils, which may extend to the periphery of the cell. Other fibrils may not insert in the centrosome, but in the granules of the microsome stratum which constitutes the boundary of the sphere, or in large granules borne upon the primary radiations. The cell granules are, in general, the insertion points of fibrils, and the granules are larger, the stronger and more numerous the fibrils that insert in them. Accordingly, the centre of the entire system — the centrosome — is the most conspicuous granule. The system of primary, secondary, and tertiary fibrils which I have described presents exactly these conditions. The fibrils lose in prominence the farther removed they are from the centre. Therefore the primary radiations are oftenest seen. The granules that give rise to secondary radiations may be called secondary centrosomes. We may thus have centrosomes of lower and lower degrees of importance until the limit is reached, — the ordinary microsome.

Reinke ('94) finds secondary and tertiary centrosomes. He advances the proposition that a centrosome is potentially present in any microsome of the cell, and that it is not an organ *sui generis*, like the nucleus. Centrosomes and microsomes, he believes, are mechanical centres. A centrosome may arise at any point when needed, by the aggregation of microsomes. Similar views were expressed by Watasé ('93). Mead ('98) shows that in the maturation of the egg of Chætopterus a large number of asters arise in the cytoplasm. They are finally reduced to two, which become the asters of the maturation spindle.

The presence of the secondary and tertiary centres in the nerve cell is not inconsistent with the belief that the primary centrosome is the centrosome left from the last mitosis. The continuity of the centrosome of mitosis with that of the resting cells has been established in many cells other than nerve cells. That a centrosome may in some cases arise *de novo* must be granted in view of the results obtained by Mead in Chætopterus. Where the centrosome is continuous it may be regarded as a permanently differentiated microsome of special functional importance. After a mitosis it persists in the resting cell and marks the starting-point of the centred system of the mature cell. As the cytoplasm of the growing cell increases, the primary centrosome becomes connected, by means of fibrils, with other granules of the new cytoplasm. With continued growth of the cytoplasm, the radiating network develops from the centre outwards, being composed of "centrosomes" and radiating fibrils of lower and lower degrees of importance according to their distance from the primary centre. This is substantially the centred system described by Heidenhain.

That the function of such a system is a mechanical one appears most probable. The centrosome is generally believed to possess an important rôle in connection with the motor activities of cells, whether of the cell as a whole (leucocytes), or of appendages of the cell (cilia; flagellum of the spermatozoön), or in the movements observed in mitosis. In the absence of known motor activities in mature nerve cells, the most likely function for the centred system is that of mechanical support.

I have found no evidence whatever that the primary centrosome of the nerve cell ever resumes its mitotic functions. In all of the regenerating worms examined, no typical nerve cells with processes were found showing any change that would suggest the possibility of their dividing.

As already mentioned (pp. 78–80), masses of dividing cells are found occasionally in the old ganglia of a regenerating worm, but no such cell proliferation is to be found in the cord of a normal worm. These cell masses probably arise from "indifferent" cells which, in the early history of the cord, ceased dividing and failed to develop into nerve cells, retaining their embryonic character. Small cells, apparently without processes and having nuclei similar to those of the nerve cells, are to be found in the normal brain and cord. The injury to the nervous system is a stimulus which may set some of these cells to dividing. The purpose of cell increase in ganglia many segments back of the region of injury is not apparent. The stimulus of the injury is not restricted to the injured segment, and, in response to it, cell proliferation may occur where it is of no direct advantage in the regeneration of the new nervous parts.

In conclusion, it is to be observed that the centrosome and the nerve process in the earthworm occupy a definite position in relation to each other and the nucleus. They are always on opposite sides of the nucleus, the centrosome occupying the greater cytoplasmic mass. The point of origin of the process may therefore be considered to be determined at as early a period as the telophase of the last mitosis in the history of the cell. The chromatic mass is the nucleus. The polar region of the spindle occupies the position of the cytoplasmic fundament. The nerve process will be developed from the equatorial side of the nucleus. In the smallest cells that give evidence of any nerve process, the centrosome and process are on opposite sides of the nucleus.

VI. The Centrosome in Cells of the Epidermis.

A study of the cells of both the old and the regenerated epidermis brings to light strong evidence for the presence of the centrosome in the resting cell of this derivative of the ectoderm. The cells of the old epidermis are less favorable for the detection of the centrosome than the regenerated cells. The old epidermis consists of much elongated columnar cells, whose contents are often coarsely granular. The cells of a recently formed epidermis are more flattened and their contents finely granular.

In Figure 43 (Plate 6) are shown two cells from the epidermis of the anterior end of a normal worm. In both of these there is a conspicuous deeply stained spherical granule, lying in a clear space directly at the deeper end of the much elongated nucleus. In the cell at the left in the figure this granule (*c'so.*) is the only prominent object in the cytoplasm. In the cell at the right, at the outer end of the nucleus, is a region in which are scattered a dozen or more deeply stained granules.

Two of them lying very close together occupy a slightly clearer space just at the outer end of the nucleus. The condition in the right cell, taken by itself, makes doubtful the interpretation of any of its granules as a centrosome. The large granule at the deeper end of the nucleus, or the pair in the small clear space at its outer end, or both, might pass for centrosomes. Or they might almost equally well be considered accidental granules of the kind which forms the group at the outer end of the nucleus. It must be noted, however, that the granule at the inner end is in some respects unique. It is larger than any of the others, its clear space is better defined, and the end of the nucleus is sharply invaginated at the region nearest the granule, the curvature of the invagination corresponding to the outline of the clear space. These facts, together with the fact that the next cell contains a similar granule, similarly located, and without the presence in the cell of any other like bodies, make it highly probable that the granule at the inner end of the nucleus of each cell is not an accidental thing.

These two cells well represent the conditions in the old epidermis. Very many, perhaps most, of the cells resemble the cell at the right in Figure 43. There are several, or very many, large granules in the cytoplasm, often at both ends of the nucleus. Frequently one of these granules lying near the inner end of the nucleus will appear slightly larger than the others, or it may lie in a clear space; yet it is not sufficiently peculiar to justify considering it anything but an accidental granule like others in the cytoplasm. The combined evidence from all such cells affords alone no conclusive argument for the presence of a centrosome. But very often there are found cells like the left one of the two in Figure 43, where there is a single conspicuous granule, which is generally situated not far from the inner end of the nucleus. In rare cases some weak radiations can be detected. It is not uncommon to find the nucleus invaginated at its inner end, as in the right cell. These granules are doubtless "centrosomes" in the sense that such bodies in resting cells are so called, without knowledge as to their history or function. The occurrence of cells with the single definitely located granule makes it likely that in cells whose cytoplasm is filled with large granules the centrosome is present, but its identity is rendered doubtful by the presence of other bodies of similar appearance.

Figures 44 and 45 represent cells from recently regenerated epidermis. A thin layer of cuticula is present. The nuclei are more nearly spherical than in the old epidermis, and the cells are more flattened. Often no distinct cell limits can be seen. These figures are from sublimate preparations stained in Kernschwartz and safranin. In Figure 44 the middle nucleus has an irregular outline on the side toward the greater cytoplasmic mass. Two small granules lie close to the nuclear membrane, each one opposite a slight concavity of the nucleus. Each granule is surrounded by a clear space. From both granules radiations extend into the cytoplasm, some of them going in straight lines nearly to the cell periphery, others (those directly opposite the nucleus, in the middle region of the cell) showing an arrangement like that described in the nerve cells. The primary radiations from the centre meet granules from which go off secondary radiations, resulting in something of a radiating network of fibres with granules at their intersections. The radiations, like those of the nerve cell, appear either as hyaline fibrils, or as rows of granules. The presence of the two central granules in Figure 44 suggests preparation for mitosis.

In Figure 45 the larger nucleus has a sharp invagination at its deeper end. Opposite the invagination is a granule with a complex radiating system about it. Primary, secondary, and tertiary radiations may be detected.

VII. Mitosis in the Regenerating Epidermis.

Figures 46–52 (Plate 7) represent stages in the mitosis of cells in the regenerating epidermis of a single worm. Figure 46 shows a cell in the prophase. The chromatin has assumed the form of nearly spherical chromosomes. The nuclear membrane is still present, but indistinct or absent at the regions where the spindle comes into relation with the chromosomes. No distinct granules could be detected at the spindle poles. Well defined polar radiations are present. The cytoplasm is fairly dense throughout the cell, and there is a distinct cell membrane. The deformation of adjoining cells shows that the cell has increased in volume. This condition is followed by the total disappearance of the nuclear membrane, and the chromosomes are assembled into an equatorial plate. Figure 47 shows the daughter chromosomes on their way toward the poles. Indistinct interzonal filaments are present. The centrosomes are extremely minute, but definite granules. Numerous polar radiations extend to the periphery throughout the entire cell.

Figure 48 shows a cell seen from the surface of the epidermis, the axis of the nucleus being perpendicular to that of the cell. The nuclear figure is in the telophase with barrel-shaped interzonal region. The centrosomes could not be found. Figures 46–52 are, with the exception of Figure 48, all from a limited region of epidermis at the ventral edge of the cut end of the worm. Figure 48 is from a mass of cells more dorsally placed, and so near the end of the alimentary tract that its epidermal origin is perhaps doubtful. No other cells in this phase could be found in the preparation.

Figures 49–51 show late telophases in the same group of cells from which Figures 46 and 47 are taken. All of these cells show a peculiar condition of the cell membrane. In Figure 49 the interzonal filaments are constricted at the equator, and there is a distinct "Zwischenkörper." An equatorial membrane has formed between the daughter cells. The triangular clearer region on the deep side of the two cells can best be interpreted as the space from which the mother-cell membrane has receded in its constricting. In the daughter cell at the right there is a narrow clearer space immediately about the interzonal filaments and the chromatic mass. On the deep side this space is so sharply outlined as to appear bounded by a membrane. In the cell at the left there is something which appears like a faint membrane (*mb.'*) lying just within the old cell membrane.

In Figure 50 there is a remarkable doubling of cell membranes, and in Figure 51 a well defined membrane (*mb.'*) in each daughter cell evidently corresponds to the membrane *mb.'* in Figure 50. Outside each of these membranes in Figure 51 is a region corresponding to the regions in Figure 50 enclosed by the membranes, *mb.* In Figure 51, however, these two regions are indefinitely outlined.

These conditions would force upon us the conclusion that a new cell membrane is formed within the old membrane of each daughter cell, and that the old membrane finally disappears. A search through the epidermis of this animal failed to reveal other stages which would throw any more light upon the question.

A centrosome may be seen in the concavity of the chromatic mass in the right daughter cell of Figure 50, and in the left daughter cell of Figure 51. In the latter figure a distinct nuclear membrane is formed about the chromatin. Figure 52 shows two sister cells from the same region. The section is somewhat oblique to the surface of the epidermis. The nuclei have increased in size, the concavity still persisting. In the cell at the right the concavity of the nucleus is filled by a dense, finely granular mass containing the centrosome with some faint radiations. The presence of the centrosome at so late a period in the history of the young cell is evidence that the central granule of the radiating system of the resting cells (Figs. 44 and 45) is a true centrosome, remaining from the last mitosis.

VIII. Mitosis in the Subepidermal Cells of the Old Epidermis.

Figures 53–56 (Plate 8) represent subepidermal cells, or basal cells of the epidermis. These cells occur wedged in between the deep ends of the columnar epidermal cells. In one worm of seven days' regeneration, many dividing cells were found among the subepidermal cells in five or six segments back of the injured segment. However, no mitosis was found in the elongated columnar epidermal cells of the same animal.

Figure 53 represents a cell in which the division is complete. The new nuclear membranes have been formed. The nuclei are cup-shaped, and the greater mass of chromatin is collected on the concave side of the nucleus. At the concavity of the nucleus in the lower cell may be seen the conical spindle region with a minute centrosome at its apex. In the upper cell there is a slightly denser region immediately outside the concavity of the nucleus, and at the centre of this denser region is the centrosome. Several indistinct granules lie at the edge of the denser region. There are some weak radiations from both centrosomes. A dividing cell membrane is present. This membrane is represented as if the cell were seen as a transparent object. The lower of the lines, *mb'*., is the intersection of the membrane with the upper surface of the old cell membrane; the upper line, *mb'*., is its deeper intersection. On the dividing membrane and in the axis of the old spindle (whose former position is marked by a darker region extending between the two nuclei) is a small dark mass, perhaps a "Zwischenkörper."

Figure 54 shows two sister cells. In one the concavity of the deeply cup-shaped nucleus, as well as a considerable space immediately outside of it, is filled by a mass of dense cytoplasm. The centrosome, an intensely staining granule with weak radiations, lies just outside the concavity of the nucleus. The condition of the young nucleus is characteristic. The chromatin is massed in a nearly solid layer on the concave side of the nucleus, while more or less isolated masses of chromatin extend from this layer to the equatorial surface of the nucleus. The upper nucleus of Figure 54 is seen in the direction of its (spindle) axis. The mass of chromatin which lines the cavity is seen as a nearly complete circular band.

A young cell whose nucleus has increased in size and lost something of its concavity is shown in Figure 55. A finely granular region separated from the outer coarser cytoplasm by a narrow clear space, and containing at its centre a prominent centrosome with some radiations, is seen at the polar concavity of the nucleus. The sister cell was immediately adjoining, but its axis was in an unfavorable position.

Figure 56 represents a cell in which the resting condition has been regained. There is still a slight flattening of the nucleus on one side, — the remains of the polar concavity. The centrosome, imbedded in a mass of cytoplasm denser and more finely granular than that of the rest of the cell, lies on the flattened side of the nucleus and directly opposite the flattened region. The sister cell was identified in the next section. It showed similar conditions as to the nucleus, slightly concave on one side, and the presence of a denser mass of cytoplasm at this region of the nucleus, but no definite centrosome could be seen.

A study of the earliest stages of regeneration may reveal that the new epidermis over the cicatrix owes its origin to the subepidermal cells rather than to the columnar cells of the old epidermis. The occurrence of mitosis in the subepidermal cells many segments back of the one injured recalls the fact of groups of actively dividing cells in ganglia remote from the cut end of the worm. The subepidermal cells may act as "Ersatzzellen," receiving an impulse toward mitosis even in segments remote from the injury.

IX. Some Peculiar Mitoses.

I desire to call attention briefly to certain dividing cells in a worm of seven days' regeneration. Directly under the new epidermis was a small mass of cells with nuclei like those of the epidermis. Abundant mitoses were found among these cells. Figure 57 shows a group of the cells with a dividing cell in the metaphase. All stages of mitosis could be found, presenting a series of conditions exhibiting no unusual features. There were telophases with chromatin solidly massed at the poles and a barrel-shaped interzonal region, the whole figure being imbedded in a mass of dense cytoplasm. Later stages showed the formation of an equatorial membrane, the constricting of the interzonal filaments and some evidence of a Zwischenkörper. The old cell membrane and the equatorial membrane eventually disappear, but at all stages there is some dense cytoplasm collected about the daughter chromatic masses. The cytoplasm of the resting nuclei is indefinitely outlined; there are no cell membranes.

Among these cells a few cases were found which exhibit exceptional conditions. These are shown in Figures 58–60. Figure 58 is the only cell of its kind which could be found. To describe it in terms of normal mitosis, there are two chromatic masses with some interzonal filaments extending in straight lines between them. Enclosing these straight fibres is a sharply defined membrane (*mb. nl.*) slightly constricted near the equatorial region. This membrane is less distinct in the neighborhood of the chromatic masses. It could not be determined whether the membrane enclosed the chromatin, or not. Outside the membrane several fibres could be seen curving from pole to pole. Figure 59 is, beyond all doubt, a later stage of the condition seen in Figure 58. The cell outlines are sharply defined, and the equatorial membrane has formed, dividing the cytoplasm. The membrane (*mb. nl.*) must be identical with the one similarly designated in Figure 58. It can now be seen to enclose the chromatin. It is deeply constricted at the equator, and some indistinct fibres extend, within this membrane, between the chromatic masses. Figure 60 is readily seen to be a stage following that of Figure 59. The division of the cytoplasm is complete, a clear space intervening between the two daughter cells. The membrane, *mb. nl.*, is very sharply constricted, and the two cells are still united by it. The chromatin is in the form of large granules. Between the two chromatic masses extend some fibres which become thicker and darker as they approach the chromatic masses.

A careful search through the series failed to reveal any other stages in this process of division, and nothing like it was seen in other worms. It can hardly be doubted that the membrane, *mb. nl.*, of Figure 60 is a nuclear membrane. The membrane, *mb. nl.*, of Figure 58 is therefore a nuclear membrane. Is it the old nuclear membrane, or a new one? The question cannot be answered with the evidence at hand. Early stages of mitosis are present in abundance, but none showed any sign of the persistence of the old nuclear membrane. However, divisions of this type are so rare that we may readily suppose its earlier stages to be absent in the preparation. If *mb. nl.* is the old nuclear membrane, we have here a case of indirect cell-division, during which the nuclear membrane persists and divides by constriction in amitotic fashion. In some Protozoa the nuclear membrane normally persists during mitosis. It has been maintained by some writers that, in ordinary mitosis, the nuclear outline persists and can be made visible by certain methods (Pfitzner, **'83**, **'86**; Waldeyer, **'88**).

The other alternative is that *mb. nl.* is a *new* nuclear membrane, formed about both chromatic masses during the early telophase and secondarily dividing by constriction. Whatever the proper explanation, the condition is of sufficient interest to invite an effort toward obtaining additional stages in the process.

X. Summary.

I. There is commonly present in the nerve cells of Lumbricidæ a centred system, consisting of centrosome and radiations.

1. The single centrosome (or rarely two, or even three, small granules lying close together) is found in the axis of the cell, on the side of the nucleus opposite the nerve process, and therefore on the side of the greatest cytoplasmic mass. It is generally not far from the nucleus and approximately at the centre of the cell as a whole.

2. Radiations consisting of fibrils bearing minute granules extend from the centrosome toward the periphery of the cell. Calling these "primary radiations," there may also be distinguished secondary radiations, which arise from certain of the larger granules in the course of the primary radiations. In rarer cases tertiary radiations may be found arising from granules in the secondary radiations. The centred system is therefore a complex one, consisting of a chief centre or centrosome, and numerous inferior centres situated throughout the cytoplasm, all with their corresponding sets of radiations, the whole system forming a radiating network whose complexity increases toward the periphery of the cell.

3. In most cases, no structure which could be called a centrosphere is present. The centrosome, as well as each of the inferior centres, is generally surrounded by a small clear space.

Sometimes the centrosome is surrounded by a narrow region of denser cytoplasm, and the primary radiations, where they intersect the periphery of this region, bear conspicuous granules. In other cases the centrosome lies in a central mass of slightly denser, more finely granular cytoplasm, of perhaps one-half the diameter of the cell, but not bounded by a layer of granules.

II. A centred system like that found in nerve cells of normal worms is found in regenerated nerve cells. Its chief centre, or centrosome, is the centrosome of the last mitosis in the history of the cell.

1. If the anterior five or ten segments of a worm be removed, the regeneration of a brain and a certain length of ventral cord (not yet segmented) takes place in the course of five weeks.

2. The epidermis is the chief source of cells which give rise to the new nervous parts.

3. In the old cord no evidence of mitosis is found among the large pear-shaped cells with processes.

4. In some cases, masses of actively dividing cells are found in ganglia many segments back of the region of injury. These masses probably owe their origin to certain small indifferent cells which have retained their embryonic character since the development of the cord.

These cell masses can take no part in the regeneration of ganglia anterior to the region of injury, except when such a mass arises at the place of injury.

The stimulus due to the injury is not restricted to the segment injured, but may cause mitosis among cells of embryonic character in ganglia remote from the region of injury.

5. The regeneration of ganglia is preceded by a forward growth of fibres from the cut end of the old cord. Cells of epidermal origin accumulate ventrally and laterally about this new fibre tract to form the fundament of the new ganglia.

The fibre bundle divides to encircle the alimentary canal. At a region dorsal to the canal, it becomes associated with a mass of cells lying dorsally and posteriorly to the fibre mass, and, together with these, constitutes the brain fundament.

6. The deeper cells of the nervous fundaments are the first to become differentiated into nerve cells. The more superficial cells long retain their embryonic character, continuing to divide actively after the deeper cells have become typical pear-shaped ganglion cells.

7. About the posterior dorsal surface of the normal brain there are some cells with scant cytoplasm, and lacking nerve processes. These are doubtless cells which, in the development of the brain, stopped dividing without becoming differentiated into nerve cells, retaining their embryonic character, perhaps for purposes of regeneration in case of injury.

8. In mitosis among cells of the nervous fundaments of about five weeks' regeneration, the following peculiarities are to be observed: —

a. Throughout the process of mitosis the body of the cell outside the limits of the spindle figure is practically homogeneous and clear.

b. A well defined cell membrane, formed during the prophase, becomes constricted in the telophase, and an equatorial membrane is formed between the daughter cells. During the later phases the old cell membrane, as well as the newly formed equatorial membrane, degenerates and disappears, leaving the two daughter chromatic masses, connected by the equatorially constricted interzonal filaments, lying free in an indefinitely outlined clear space.

c. The daughter chromosomes, having completed their migration toward the poles, fuse to form solid chromatic masses, concave on their polar sides. *The chromatic mass represents the nucleus.*

d. In the concavity of each chromatic mass is a sharply outlined, lightly stained, conical region, — the region of the old spindle-end, — with the centrosome at its apex. *This conical polar region is the fundament of the cytoplasm of the young cell.*

e. The interzonal filaments disappear, leaving the two chromatic masses and their accompanying polar structures lying free in a common, irregularly defined, clear space formerly occupied by the mother cell. *All the living parts of each daughter cell are comprised within the chromatic mass and its polar region.*

f. The two young sister cells may become separated by the pushing in of adjacent tissue between them.

g. The transformation of the chromatic mass into a resting nucleus involves the swelling of the newly formed nuclear membrane, the increase in the volume of its contents probably being due to the absorption of fluid material. The solid mass of chromatin meanwhile gradually breaks up into small granules which, together with the achromatic substance, form the loose peripheral nuclear network of the resting nucleus. During this process a nucleolus appears.

h. Accompanying these changes in the nucleus, the polar cytoplasmic fundament increases in volume, remaining always sharply outlined in the surrounding clear space. For a considerable period the growing cytoplasm is massed upon the concave polar side of the nucleus. At length the nuclear concavity disappears and the increasing cytoplasm envelops the entire nucleus. By far the greater mass of cytoplasm, however, always remains on the polar side of the nucleus.

i. The size and form of the typical pear-shaped nerve cell are finally attained as a result of the continued swelling of the nucleus, the increase in volume of its large polar body of cytoplasm, and the development of a nerve process from the smaller mass of cytoplasm on the equatorial side of the nucleus.

j. Distinct centrosomes are to be seen at the poles of the mitotic figure at all stages.

After the disappearance of the interzonal filaments the centrosome is found at the apex of the polar cytoplasmic fundament, attaining its greatest size at this period.

During the early growth of the cytoplasm the centrosome persists, generally being found not far from the centre of the cytoplasmic mass. At an early period evidences of concentric and radiating structure about the centrosome are seen.

With continued increase of the cytoplasm, the centrosome assumes its characteristic position in the axis of the cell near the nucleus, and becomes associated with a system of radiating fibres whose complexity increases until the conditions found in the mature cell (I. 2) are attained.

III. 1. Some resting cells of recently regenerated epidermis possess a centrosome and system of radiations similar to those of the nerve cells.

2. In the mitosis of cells in the regenerating epidermis the centrosome persists in the cytoplasm after the nucleus has regained the resting condition.

3. In the much elongated columnar cells of the epidermis of the normal worm, there is strong evidence for the presence of a centrosome. In these very attenuated cells, however, no radiating system could be detected.

4. The final stages of mitosis in some cells of the regenerating epidermis exhibit peculiar conditions of the cell membranes, pointing toward the conclusion that, after the division of the cytoplasm, a new cell membrane forms within the old cell membrane of each daughter cell, the original cell membrane disappearing.

IV. 1. The stimulus due to the injury may cause abundant mitosis among the subepidermal cells or basal cells of the epidermis, several segments back of the injured segment.

2. The centrosome of mitosis in these subepidermal cells persists in the cytoplasm after the cell has returned to the resting condition.

V. In certain cicatricial cells, evidently of epidermal origin, a few mitoses were found where, in the telophases, both daughter chromatic masses were enclosed within a common membrane of doubtful origin (a distinct outer cell membrane being also present). This membrane finally constricts and divides equatorially, becoming, apparently, the nuclear membrane of the two daughter nuclei.

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EXPLANATION OF PLATES.

All the figures were drawn with the aid of the camera lucida. Unless otherwise stated, all preparations were fixed in Flemming's stronger chromic-osmic-acetic mixture.

Owing to defective printing, centrosomes and chromatic granules sometimes appear as rings, instead of solid bodies.

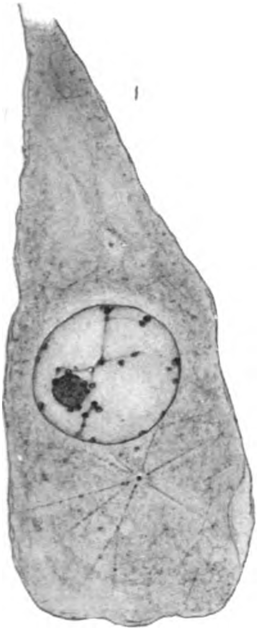
ABBREVIATIONS.

a.	Anterior.
can.ali.	Alimentary canal.
chr.	Chromatic mass (nucleus).
cic.	Cicatrix (cicatricular tissue).
cl. mit.	Mitotic cell.
cl. pr'f.	Mass of proliferating cells.
c'so.	Centrosome.
cta.	Cuticula.
d.	Dorsal.
ec'drm.	Old epidermis.
ec'drm'.	Regenerated epidermis.
gn.	Ganglion.
gn. nov.	Fundament of ganglia.
gn. su'œs.	Fundament of brain.
mb.	Mother-cell membrane.
mb'.	New cell membrane.
mb. nl.	Nuclear membrane.
mu. crc.	Old circular muscles.
mu. crc'.	Regenerated circular muscles.
mu. lg.	Old longitudinal muscles.
mu. lg'.	Regenerated longitudinal muscles.
reg. pol.	Polar region; cytoplasmic fundament.
stmd.	Stomodæum.
te.	Testis.
trt. fbr.	Regenerated fibre tract.
tu.	Sheath of nerve cord.
tu'.	Cut end of old sheath.
*	In Figures 14–16, the * marks the point where the old epidermis ends and the regenerated epidermis begins.

PLATE 1.

All the figures magnified 2000 diameters.

Figs. 1–3. Cells from the subœsophageal ganglion of a normal worm. Iron-hæmatoxylin.
Fig. 4. Cell from the brain of the same worm.
Figs. 5, 6. Cells from a posterior ganglion of a normal worm. Acetic-sublimate, iron-hæmatoxylin.



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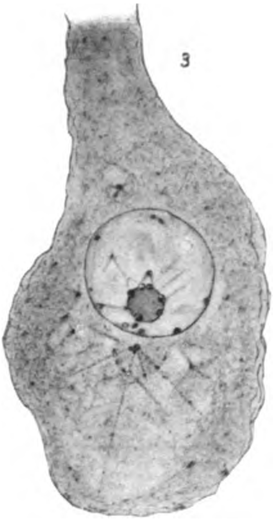

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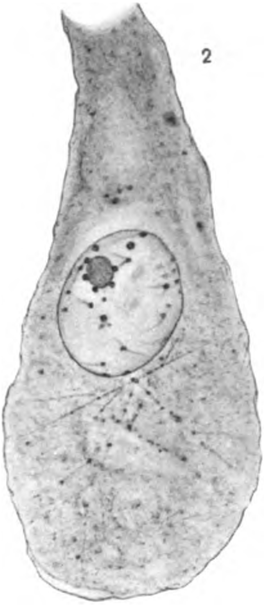

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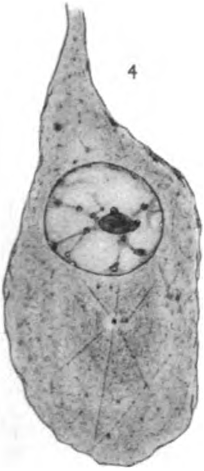

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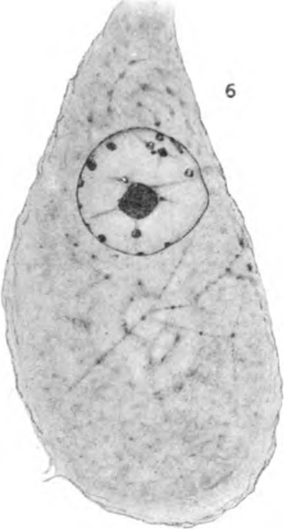

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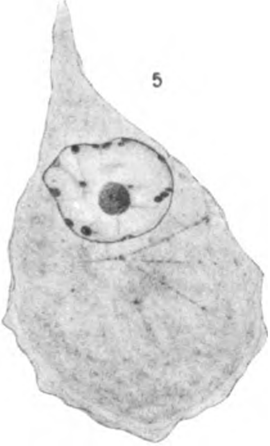

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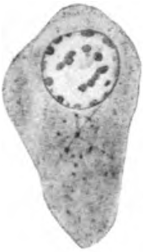
PLATE 2.

All the figures magnified 2000 diameters.

Figs. 7–9.	Smaller cells from the posterior dorsal region of the brain of a normal worm. Iron-hæmatoxylin.
Figs. 10–12.	Cells from a regenerated brain after 34 days' regeneration. These three cells lay side by side in the same section. Iron-hæmatoxylin.
Fig. 13.	Cell from a brain of 34 days' regeneration. Iron-hæmatoxylin.

7



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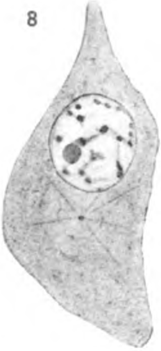

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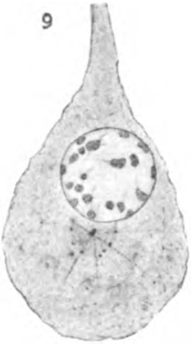

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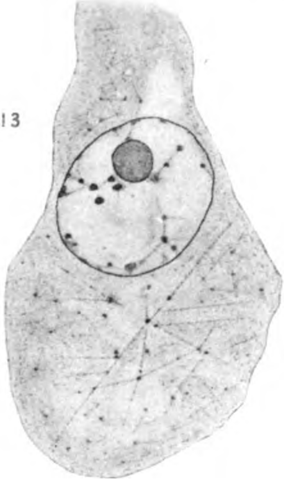

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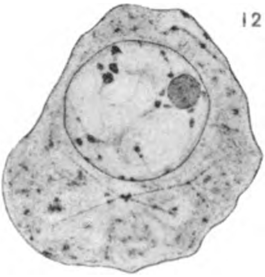

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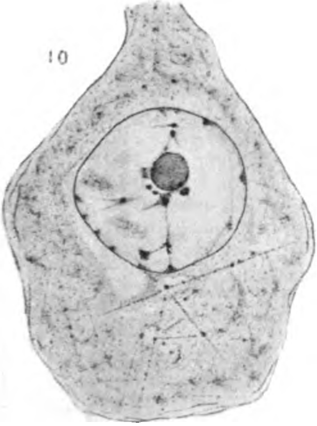

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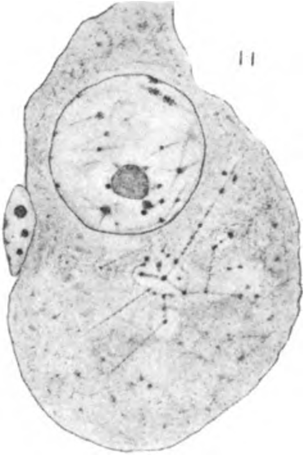


PLATE 3.

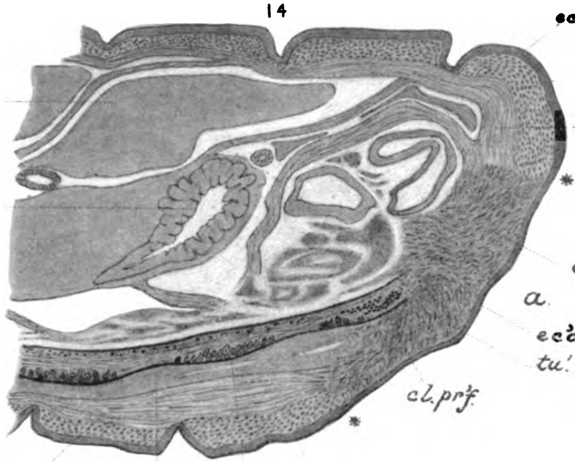
Fig. 14. Parasagittal section through the anterior end of a worm after 7 days' regeneration. × 25. Hermann's plat.-acet.-osm., iron-hæmatoxylin.

Fig. 15. Parasagittal section through the anterior end of a worm after 16 days' regeneration. × 25. Acetic-sublimate, iron-hæmatoxylin.

Fig. 16. Parasagittal section through the anterior end of a worm after 24 days' regeneration. × 25. Acetic-sublimate, iron-hæmatoxylin.

Fig. 17. Spindle cells from the cicatrix of the worm from which Figure 14 is taken. (7 days' regeneration.) × 2000.

14



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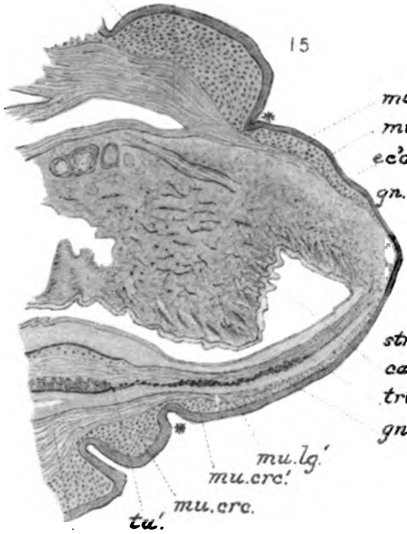

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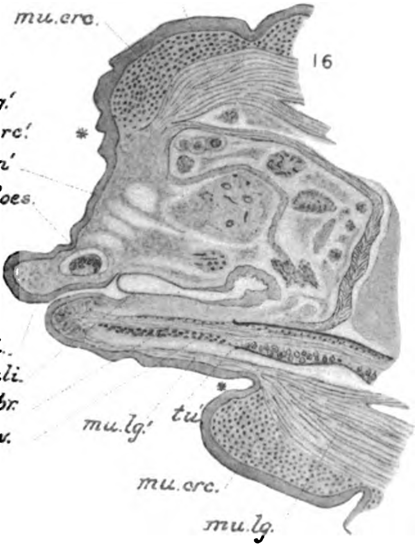

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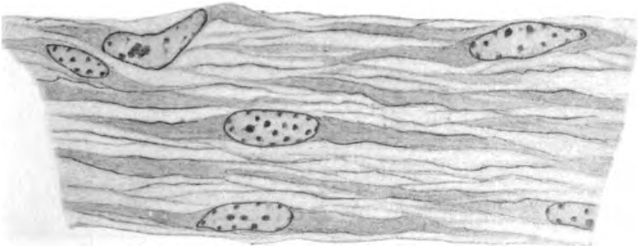


H.W.R. del.

PLATE 4.

Fig. 18. Anterior end of the nerve cord of Figure 14, showing a mass of newly formed cells. (7 days' regeneration.) × 160.

Fig. 19. Parasagittal section through the middle region of one lobe of the brain of a normal worm. × 160.

Fig. 20. Parasagittal section through the middle region of one lobe of a brain of 34 days' regeneration. × 160.

Fig. 21. A dividing cell in the prophase and one in the metaphase (one pole cut away) from a brain of 34 days' regeneration. × 2000. Iron-hæmatoxylin.

Fig. 22. Metaphase from a brain of 34 days' regeneration. × 2000. Iron-hæmatoxylin.

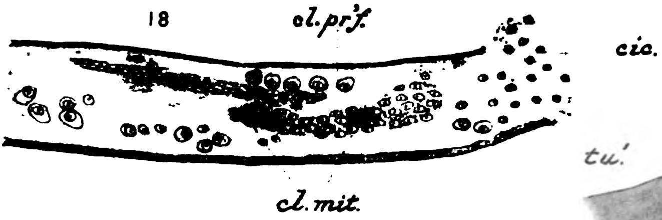
Fig. 23. Metaphase from the anterior end of a cord after 34 days' regeneration. × 2000. Gentian violet.

Fig. 24. Metaphase from a brain of 34 days' regeneration. × 2000. Gentian violet.

Fig. 25. Telophase from a brain of 34 days' regeneration. × 2000. Gentian violet.

Fig. 26. Telophase from the posterior dorsal region of a brain of 34 days' regeneration. × 2000. Iron-hæmatoxylin.

18



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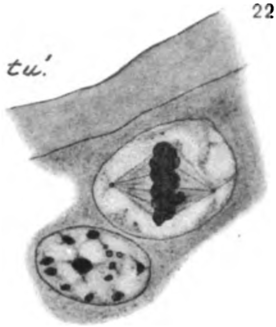

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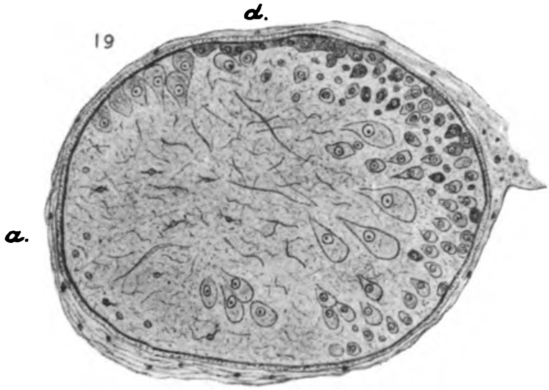

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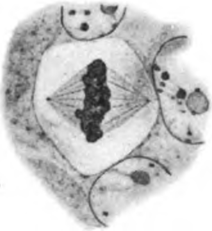

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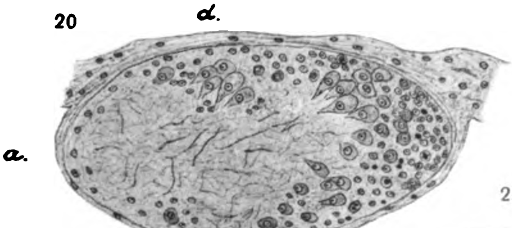

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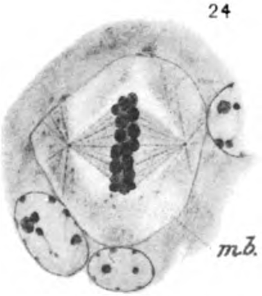

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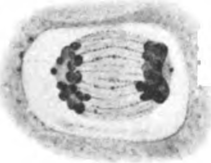

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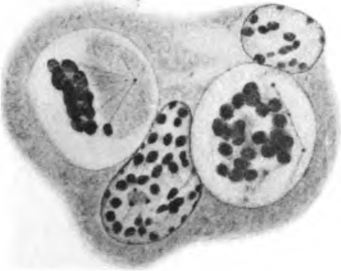

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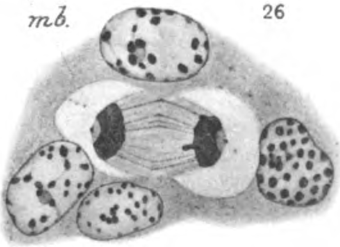

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PLATE 5.

All the figures magnified 2000 diameters.

Fig. 27. Telophase from a brain of 34 days' regeneration. Gentian violet.

Fig. 28. Telophase from a brain of 34 days' regeneration. One chromatic mass cut away. Gentian violet.

Fig. 29. Telophase from a brain of 34 days' regeneration. Reconstructed from two sections. Above it, a young cell. Iron-hæmatoxylin.

Fig. 30. Telophase from the anterior end of a cord after 34 days' regeneration. Gentian violet.

Fig. 31. Young cell (with several of the neighboring nuclei) from the posterior ventral region of a brain of 34 days' regeneration. Iron-hæmatoxylin.

Figs. 32*a*, 32*b*. A pair of young sister cells from a brain of 34 days' regeneration. The cells are in successive sections. Iron-hæmatoxylin.

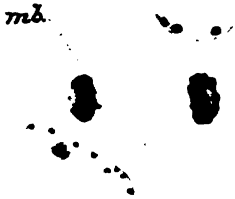
Fig. 33. Two pairs of young sister cells from a brain of 34 days' regeneration. α and β are one pair. The two cells of the other pair lie one over the other. γ is the upper of the two and the outline of the lower is indicated by δ. Gentian violet.

Figs. 34, 35. Young cells from the anterior end of a cord of 34 days' regeneration. Iron-hæmatoxylin.

Fig. 36. A group of cells from the posterior ventral region of a brain of 34 days' regeneration. At the centre of the group is a young cell. Iron-hæmatoxylin.

Fig. 37. Young cell from the dorsal region of a brain of 34 days' regeneration. Gentian violet.

27



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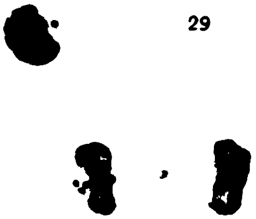

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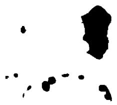

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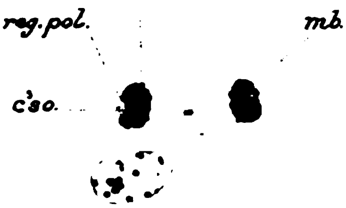

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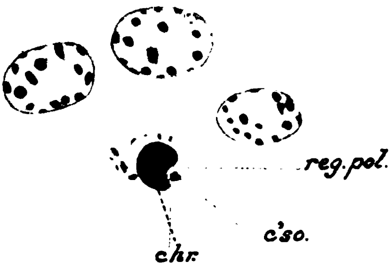

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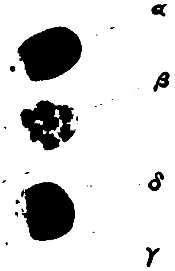

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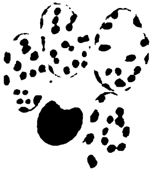

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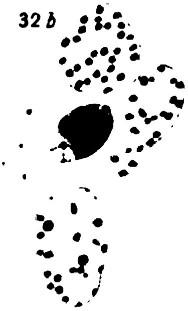

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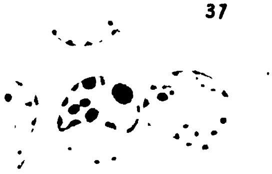

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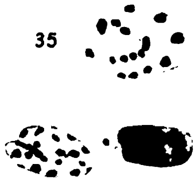

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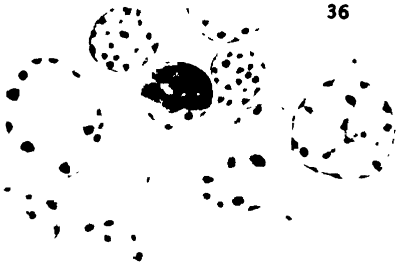

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PLATE 6.

All the figures magnified 2000 diameters.

Fig. 38. Young cell (with several of the neighboring nuclei) from the anterior end of a cord of 34 days' regeneration. Iron-hæmatoxylin.

Fig. 39. Small cells with resting nuclei from the anterior end of a cord of 34 days' regeneration. Each of the two cells has a centrosome. Iron-hæmatoxylin.

Fig. 40. Cell with a greater mass of cytoplasm than in the cells of Figure 39; centrosome and radiations. From a brain of 34 days' regeneration. Iron-hæmatoxylin.

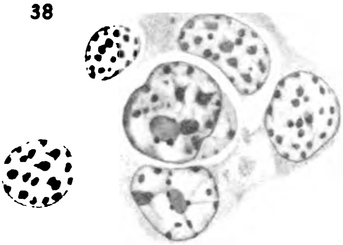
Fig. 41. Cell, similar to that of Figure 40, from a brain of 37 days' regeneration; centrosome and radiations. Iron-hæmatoxylin.

Fig. 42. Three cells from the anterior end of a cord of 34 days' regeneration. A centrosome with radiations may be seen near each of the two larger nuclei, on the side toward the greatest cytoplasmic mass. Iron-hæmatoxylin.

Fig. 43. Two cells from the epidermis of a normal worm. The centrosome is at the deeper end of each nucleus. Iron-hæmatoxylin.

Figs. 44, 45. Cells from regenerated epidermis, showing centrosomes and radiations. 16 days' regeneration. Acetic-sublimate, Kernschwartz and safranin.

38



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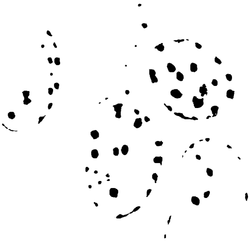

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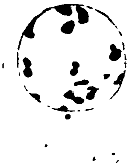

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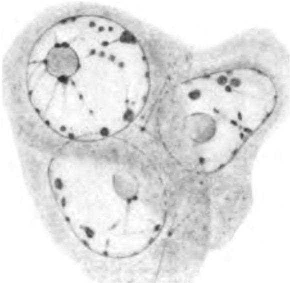

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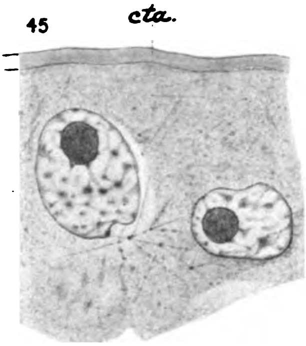

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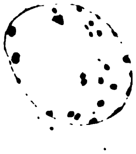

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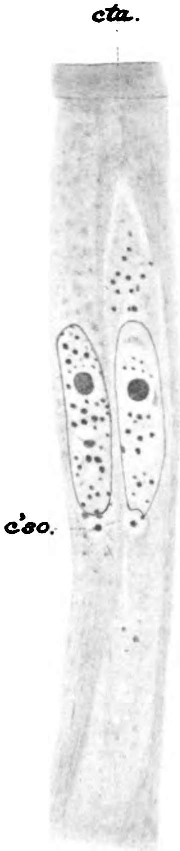

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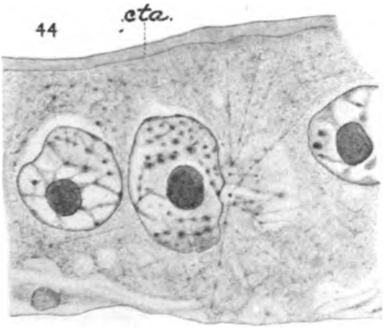

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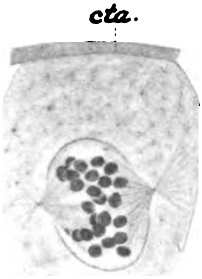
PLATE 7.

All the figures magnified 2000 diameters.

Figs. 46–52. Dividing or recently divided cells from the regenerated epidermis of a worm, after 11 days' regeneration. Hermann's plat.-acet.-osm., iron-hæmatoxylin. See pages 146–147 for a discussion of the membranes.

46



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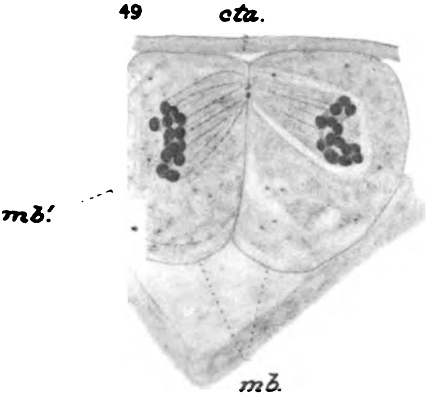

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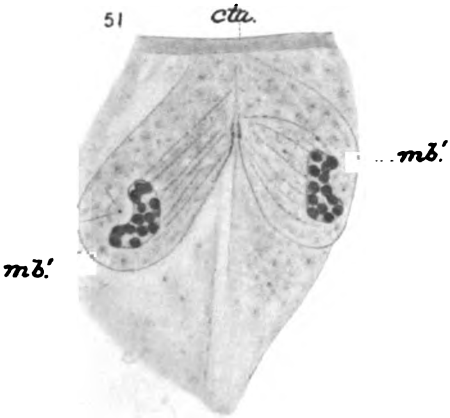

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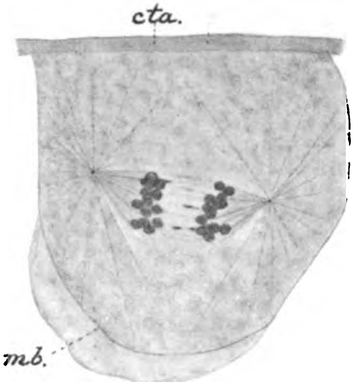

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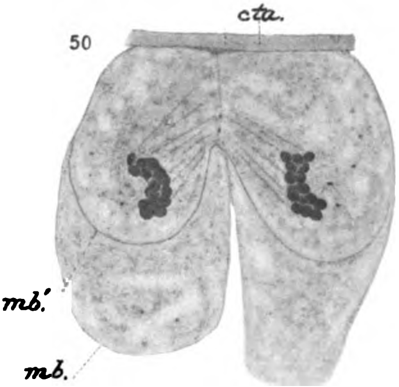

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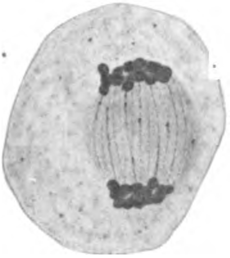

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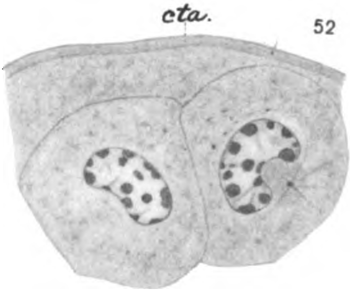

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PLATE 8.

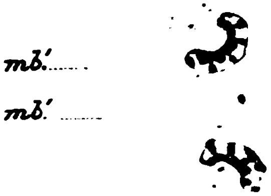
All the figures magnified 2000 diameters.

Figs. 53–56. Recently divided subepidermal or basal cells of the old epidermis, from one to four segments back of the segment cut. The centrosome of mitosis persists in the resting cells. 7 days after the operation. Hermann's plat.-acet.-osm., iron-hæmatoxylin.

Fig. 57. Group of cells, with nuclei of the epidermal type, from a thin layer of cells lying directly beneath the regenerated epidermis of a worm of 11 days' regeneration. Hermann's plat.-acet.-osm., iron-hæmatoxylin.

Figs. 58–60. Dividing cells from the layer of cells mentioned in the explanation of Figure 57, showing peculiar conditions as to the presence of a nuclear membrane. See page 149.

53



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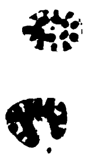

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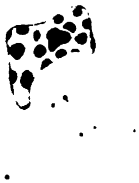

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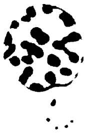

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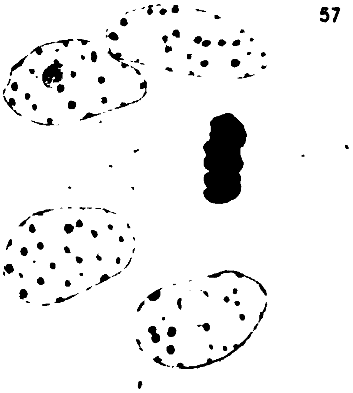

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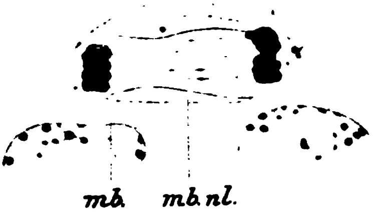

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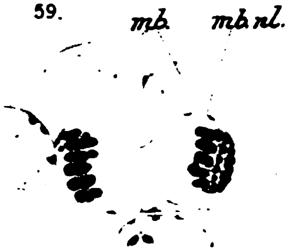

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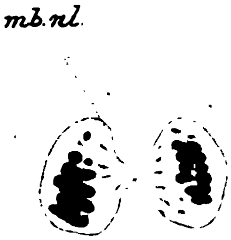

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE.

E. L. MARK, *Director*.

⁂ Abbreviations used:—

B. M. C. Z. . . . . for Bull. Mus. Comp. Zoöl.
P. A. A. . . . . for Proceed. Amer. Acad. Arts and Sci.
P. B. S. N. H. . . . . for Proceed. Bost. Soc. Nat. Hist.

1. **Barnes**, W.—On the Development of the Posterior Fissure of the Spinal Cord, and the Reduction of the Central Canal, in the Pig. P. A. A. **19**: 97–110. 3 pls. 1884.
2. **Tuttle**, A. H.—The Relation of the External Meatus, Tympanum, and Eustachian Tube to the First Visceral Cleft. P. A. A. **19**: 111–132. 2 pls. 1884.
3. **Ayers**, H.—On the Development of Oecanthus niveus and its Parasite, Teleas. Mem. Bost. Soc. Nat. Hist. **3**: 225–281. 8 pls. Jan., 1884.
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5. **Patten**, W.—The Development of Phryganids, with a Preliminary Note on the Development of Blatta Germanica. Quart. Journ. Micr. Sci. **24**: 549–602. 3 pls. 1884.
6. **Reighard**, J.—On the Anatomy and Histology of Aulophorus vagus. P. A. A. **20**: 88–106. 3 pls. Oct., 1884.
7. **Faxon**, W.—Descriptions of New Species of Cambarus; to which is added a Synonymical List of the Known Species of Cambarus and Astacus. P. A. A. **20**: 107–158. Dec., 1884.
8. **Locy**, W.—Observations on the Development of Agelena naevia. B. M. C. Z. **12**: 63–103. 12 pls. Jan., 1886.
9. **Fewkes**, J. W.—Report on the Medusae collected by the U. S. Fish Commission Steamer Albatross in the Region of the Gulf Stream in 1883-'84. Ann. Rep. Comnr. Fish and Fisheries for 1884, 927–980, 10 pls., 1886.
10. **Ayers**, H.—On the Carapax and Sternum of Decapod Crustacea. Bull. Essex Inst. **17**: 49–59. 2 pls. 1886.
11. **Mark**, E. L.—Simple Eyes in Arthropods. B. M. C. Z. **13**: 49–105. 5 pls. Feb., 1887.
12. **Parker**, G. H.—The Eyes in Scorpions. B. M. C. Z. **13**: 173–208. 4 pls. Dec., 1887.
13. **Mayo, Florence**.—The Superior Incisors and Canine Teeth of Sheep. B. M. C. Z. **13**: 247–258. 2 pls. Jun., 1888.
14. **Platt, Julia** B.—Studies on the Primitive Axial Segmentation of the Chick. B. M. C. Z. **17**: 171–190. 2 pls. Jul., 1889.
15. **Mark**, E. L.—Studies on Lepidosteus. Part 1. B. M. C. Z. **19**: 1–127. 9 pls. Feb., 1890.
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18. **Ayers**, H.—The Morphology of the Carotids, based on a Study of the Blood-vessels of Chlamydoselachus anguineus, Garman. B. M. C. Z. **17**: 191–223. 1 pl. Oct., 1889.
19. **Davenport**, C. B.—Cristatella: The Origin and Development of the Individual in the Colony. B. M. C. Z. **20**: 101–161. 11 pls. Nov., 1890.
20. **Parker**, G. H.—The Eyes in Blind Crayfishes. B. M. C. Z. **20**: 153–162. 1 pl. Nov., 1890.

21. **Henchman, Annie P.** — The Origin and Development of the Central Nervous System in Limax maximus. B. M. C. Z. **20**: 169–208. 10 pls. Dec., 1890.

22. **Ritter, W. E.** — The Parietal Eye in some Lizards from the Western United States. B. M. C. Z. **20**: 209–228. 4 pls. Jan., 1891.

23. **Davenport, C. B.** — Preliminary Notice on Budding in Bryozoa. P. A. A. **25**: 278–282. Mar., 1891.

24. **Woodworth, W. M.** — Contributions to the Morphology of the Turbellaria. — I. On the Structure of Phagocata gracilis, Leidy. B. M. C. Z. **21**: 1–44. 4 pls. Apr., 1891.

25. **Parker, G. H.** — The Compound Eyes in Crustaceans. B. M. C. Z. **21**: 45–142. 10 pls. May, 1891.

26. **Ward, H. B.** — On some Points in the Anatomy and Histology of Sipunculus nudus, L. B. M. C. Z. **21**: 143–184. May, 1891.

27. **Field, H. H.** — The Development of the Pronephros and Segmental Duct in Amphibia. B. M. C. Z. **21**: 201–342. 8 pls. Jun., 1891.

28. **Davenport, C. B.** — Observations on Budding in Paludicella and some other Bryozoa. B. M. C. Z. **22**: 1–114. 12 pls. Dec., 1891.

29. **Smith, F.** — The Gastrulation of Aurelia flavidula, Pér. and Les. B. M. C. Z. **22**: 115–126. 2 pls. Dec., 1891.

30. **Johnson, H. P.** — Amitosis in the Embryonal Envelopes of the Scorpion. B. M. C. Z. **22**: 127–162. 3 pls. Jan., 1892.

31. **Boyer, E. R.** — The Mesoderm in Teleosts: especially its Share in the Formation of the Pectoral Fin. B. M. C. Z. **23**: 91–134. 8 pls. Apr., 1892.

32. **Ward, H. B.** — On Nectonema agile. B. M. C. Z. **23**: 135–188. 8 pls. Jun., 1892.

33. **Davenport, C. B.** — On Urnatella gracilis. B. M. C. Z. **24**: 1–44. 6 pls. Jan., 1893.

34. **Davenport, C. B.** — Note on the Carotids and the Ductus Botalli of the Alligator. B. M. C. Z. **24**: 45–50. 1 pl. Jan., 1893.

35. **Ritter, W. E.** — On the Eyes, the Integumentary Sense Papillae, and the Integument of the San Diego Blind Fish (Typhlogobius californiensis, Steindachner). B. M. C. Z. **24**: 51–102. 4 pls. Apr., 1893.

36. **Nickerson, W. S.** — The Development of the Scales in Lepidosteus. B. M. C. Z. **24**: 115–140. 4 pls. Jul., 1893.

37. **Davenport, C. B.** — Studies in Morphogenesis. — I. On the Development of the Cerata in Æolis. B. M. C. Z. **24**: 141–148. 2 pls. Jul., 1893.

38. **Woodworth, W. McM.** — A Method of Orienting small Objects for the Microtome. B. M. C. Z. **25**: 45–47. Dec., 1893.

39. **Kofoid, C. A.** — On some Laws of Cleavage in Limax. P. A. A. **29**: 180–203. 2 pls. 1894.

40. **Davenport, C. B.** — Studies, etc. — II. Regeneration in Obelia and its Bearing on Differentiation in the Germ-Plasma. Anat. Anz. **9**: 283–294. 6 figs. Feb. 15, 1894.

41. **Holbrook, A. T.** — The Origin of the Endocardium in Bony Fishes. B. M. C. Z. **25**: 75–97. 5 pls. Aug., 1894.

42. **Castle, W. E.** — On the Cell Lineage of the Ascidian Egg. A Preliminary Notice. P. A. A. **30**: 200–216. 2 pls. Oct., 1894.

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COLLEGE. E. L. MARK, DIRECTOR.

No. 127.

STUDIES ON THE REACTIONS OF LIMAX MAXIMUS TO DIRECTIVE STIMULI.

BY PETER FRANDSEN.

WITH TWENTY-TWO FIGURES.

FROM THE PROCEEDINGS OF THE AMERICAN ACADEMY OF
ARTS AND SCIENCES, VOL. XXXVII. NO. 8.

CAMBRIDGE, MASS., U. S. A.
OCTOBER, 1901.

Proceedings of the American Academy of Arts and Sciences.

VOL. XXXVII. No. 8.—OCTOBER, 1901.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR.—No. 127.

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Presented by E. L. Mark. Received September 3, 1901.

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INTRODUCTION.

THE following studies were made at Harvard University during the fall and winter of 1898–99. The problem was proposed by Dr. C. B. Davenport and the investigation carried on under his immediate direction. I wish here to acknowledge my indebtedness to his many suggestions and helpful criticisms throughout the year. In connection with the preparation of the manuscript for publication, I am under obligation to Dr. E. L. Mark for many kindnesses.

The behavior of any organism toward artificial stimulation is probably always largely dependent on its normal environmental conditions. The long action of those conditions, assisted, perhaps, by the animal's own efforts, conscious or unconscious, to adapt itself to them, finally results in certain habits and instincts. The process of adaptation being extremely slow, organisms are strongly averse to great or sudden changes in their environment and incapable of adjusting themselves to them. As a rule, then, we should expect animals to seek those conditions of light, heat, moisture, and other physical and chemical influences which are most in accordance with those to which they are normally subjected.

The most easily observed responses of animals are naturally those which find their expression in locomotion. The number of stimuli which may influence locomotion are, of course, numerous, but of these a certain limited number play much the larger part. If we had an accurate knowledge of the relative weight of these different forces, we might predict with certainty the path any animal would follow under certain given conditions. An experimental study of the different stimuli ought at least to enable us to find out which ones do operate, and perhaps to establish certain general laws regarding them and the biological tendencies which impel the animal to respond.

The present paper is a study of the locomotor responses of the slug Limax maximus to three kinds of stimuli, — those of touch, gravity, and light. In connection with these studies new problems have constantly arisen, some of which have been cursorily considered, many others merely alluded to, so that the work is far from being complete.

The term "geotaxis" has been used to designate the influence of gravity on locomotion. Interesting and careful studies have been made on the geotaxis of numerous Protista by Schwarz ('84), Aderhold ('88), Massart ('91), and Jensen ('93). These investigations clearly show a geotactic response in the unicellular organisms studied. The kind of response varies according to other conditions, such as those of light, heat, density of medium, chemical influences, etc., and may also differ in individuals of the same genus under apparently like conditions. Massart ('91, pp. 161–162) found that, when a number of Spirilla were put into a vertical tube, one group collected in the upper part and another at the lower part. He also found (p. 164) that Chromulina woroniniana was negatively geotactic — that is, moved upward, or in a direction opposite to that of the pull of gravity — at 15° to 20° C., but positively geotactic at 5° to 7° C. Jensen's work also showed the important influence of other agents in modifying geotaxis. Loeb ('88, pp. 7–8) found that cockroaches preferred the steepest side of a box whose four sides were inclined at different angles; that is, they are negatively geotactic. He also discovered that a number of other Metazoa were geotactic.

In a certain way, the present paper is a continuation of a recent study made by Dr. C. B. Davenport and Miss Helen Perkins on geotaxis in the slug. Davenport and Perkins ('97, p. 105) discovered that the intensity of the animal's geotactic response was directly proportional to the sine of the angle of deviation from the vertical, and hence "varied directly as the active component of gravity." In the third section of their paper, the question, "What determines whether the head end of the slug shall be directed up or down?" was raised and considered. The results showed that certain individuals appeared to have a fairly marked positive geotaxis, for, when placed on an inclined glass plate, such animals swung the head-pole of the axis toward the earth; but others showed as strongly marked a tendency to move away from the earth, and a few seemed indifferent as to whether they went up or down. Their experiments showed further that there was, apparently, no inherent tendency in individual animals to move either to the right or to the left, so that the difference in geotactic response could not be explained as due to differences of an inherent tendency of this kind. The effect of a slight initial impulse given to the head of the animal indicated that the thigmotactic, or contact, stimulus imparted to the animal in handling might, to some extent, modify its response to the stimulus of gravity. But Davenport and Perkins did not reach any definite, satisfactory answer to the main question.

It was to test their observations by a larger number of experiments, and, if confirmed, to explain them by further experimentation, that the present investigation was undertaken. In the first place, I wished to find out whether certain individuals, if put on an inclined glass plate, always responded to the pull of gravity by directing the head end up and moving away from the earth, and whether certain other individuals always did the contrary. If this proved to be true, then it was my main problem to seek the reason for it. Is the force which makes some slugs go up, others down, and still others indifferent to the attraction of gravity, a purely accidental one, — is it a physical force, or is it what we may call a psychical peculiarity, which varies in different individuals and in the same individual at different times? As a preliminary to the main problem, I first made a series of experiments on the animal's thigmotaxis, — its response to contact- and pressure-stimuli. By virtue of its thigmotaxis, an animal moves either toward or away from the agent which comes in contact with it, just as its geotaxis is expressed in a movement toward or away from the earth, in response to the attraction of gravity.

I. Thigmotaxis.

Material and Methods. — The animal used in all the following experiments was Limax maximus, which is fairly abundant in the greenhouses about Cambridge. Material was obtained from several different greenhouses and kept in a large closed tin box, the bottom of which was covered with moss kept moist, so as to afford an environment as much like the customary one as possible. Fresh cabbage leaves constituted the animal's main food. The cannibalistic tendencies of the slug, together with an unavoidable deterioration due to repeated handling, necessitated a frequent renewal of the animals.

The methods used in the experiments were simple. The slug was placed on a circular glass plate set horizontally in the bottom of a cuboidal wooden box which was made impervious to light and covered with a thick, black cloth. Precautions were taken to avoid thermal and chemical influences by keeping the box at as equable a temperature as possible and by wiping the plate free from slime before each test. The tests were made only when the animal had definitely oriented itself and was moving ahead in a straight line. Two series were made. In the first series the dorsal tentacle was touched gently with the forefinger. The box was then immediately covered with the black cloth. Observations were made after the lapse of 20 to 30 seconds and the position of the animal noted. The right and left tentacles were touched alternately.

Results. — The following Table (I.) gives the results of a number of experiments on ten different animals.

TABLE I.

RESPONSE TO THIGMOTACTIC STIMULATION OF THE TENTACLES.

Animal No.	Number of Trials.			Total Number of Trials.
	–	+	0	
1	7	2	3	12
2	11	3	3	17
3	8	3	1	12
4	4	3	3	10
5	7	2	0	9
6	6	0	2	8
7	10	2	2	14
8	16	4	2	22
9	18	1	5	24
10	22	1	4	17
Totals . .	99	21	25	145

The column headed with the minus sign shows the number of times the animal responded by moving *away* from the source of stimulus; the one headed with the plus sign, the number of times it moved *towards* that source; and the zero column, the number of times there was *no* response. I found that the animal would respond very definitely and precisely to stimuli two or three times in succession by immediately retracting the tentacle touched and moving away from the stimulating influence. After the third trial, however, it either refused to change its direction of locomotion or else moved directly towards the source of the stimulus. If a respite of a few seconds before the next stimulation was then permitted, the animal would again give a precise negative response for two or three trials, and then, as before, it desisted. Out of the total 145 tests, there was a negative response in two thirds of the trials. The remaining trials — one third of the whole — were about equally divided between the positive responses and refusals to respond at all. Sometimes five or six tests were made in quick succession, so that the total negative response is rather less than it would have been if a rest had been given in each case after three tests. Out of the 21 cases of direct positive response, 15 were cases where the right tentacle was touched, and the remaining 6 were due to stimulation of the left tentacle. Similar, but more marked, differences between the results of stimulating the right and the left tentacles were observed in other experiments. This suggests that either the right tentacle may be less sensitive to stimuli, or that its counterirritancy may be more readily aroused. There is, however, a third possible cause. The animal may have an innate tendency to go to the right, and, if so, this tendency may diminish to some extent the force of the stimulating agent when it impinges on the right side of the animal, and correspondingly increase the response when the stimulus is directed upon the left side of the animal. Something further will be said about this point in a later part of the paper.

A few thigmotactic experiments were next made on the sides of the animal posterior to the head. The right and left sides were touched alternately. The results are given in Table II.

Phenomena like those observed in stimulating the tentacles are seen here, and they also agree with similar observations by Davenport and Perkins ('97, p. 109.) After two or three trials, the animal begins to show resistance, and if the finger is held against its side, will sometimes try to displace the finger by pushing against and curling the body around it. The frequency of the negative response is here somewhat less marked than in the preceding experiments, which is as we should expect, owing to the greater sensitiveness of the tentacles as special tactile organs. In these experiments every one of the minus and zero results was due to stimulation of the right tentacle.

TABLE II.

RESPONSE TO THIGMOTACTIC STIMULATION OF THE SIDES OF THE BODY.

Animal No.	Number of Trials.			Total Number of Trials.
	−	+	0	
1	11	3	3	17
2	8	6	2	16
3	17	5	4	26
Totals ..	36	14	9	59

These facts clearly prove that, under ordinary circumstances, the slug is negatively thigmotactic. In our consideration of the animal's responses to other stimuli, we shall have to take this into account, as causing occasional vagaries, and therefore endeavor to eliminate it as much as possible from the experiments.

II. GEOTAXIS.

What determines whether the head end of the slug shall be directed up or down?

Methods. — The same apparatus was used as in the preceding experiment. A circular glass plate was employed so that the animal could be rotated into any desired position without the necessity of its being handled. The plate was set in a box at an angle of about 45° with the horizon. In each test the animal was so placed on the plate that the long axis was horizontal, different sides being directed downward in different trials. At first the experimentation consisted mostly of watching the animals in order to obtain some clue for further work. Later, rough sketches of the pigment patterns of the individual animals were made, so that it was possible to identify individuals with certainty; the same animal could then be subjected to experiments at different times and the difference in results noted. The methods used in working out particular questions will appear as these questions are considered. As the same number of experiments were not made on each animal studied, I have, for the sake of comparison, estimated in each case the geotaxis in per cents. This percentage is obtained by dividing the number of positive or negative responses by the total number of responses. The nearer the geotaxis percentage approaches 100 the more precise has been the kind of response. No fixed time was allowed to elapse between successive tests, but in each test the observation was made at an interval of from 30 to 60 seconds after covering the box.

Operations and Results. — The first question investigated was whether particular animals exhibited a decisive positive or negative geotaxis. A number of tests were, therefore, made on each of several selected individuals. The results obtained were like those of Davenport and Perkins ('97, p. 108); that is, certain animals showed a very marked positive geotaxis; others, an equally decided negative tendency; and a few, perhaps one animal out of 12 or 15 where 10 or more tests were made, were apparently geotactically indifferent. The occasional irregularities in the responses of individual animals were easily seen to be due to influences other than pure gravity, such as jarrings of the plate, influence of contact in putting the animal on the plate, and to the influence of light admitted in lifting the cover of the box. Frequently, upon the raising of the cloth to make an observation, the animal would retract its tentacles, as if dazzled by the sudden inflow of light, and at the next observation it would be seen to have altered its response.

Naturally, this question next arose, Is the response the same on different days? In Table IV. (p. 195) are given the results with a number of animals experimented on to test this point. These are numbers 2, 7, 8, 22–25, 27. Number 2 was positively geotactic on two days and negative on another day. A similar variation is seen in the case of slugs 7 and 22. In the case of all the rest, however, there is a very marked constancy. The ninth (last) column in the table indicates the condition of the animals at the time of experimentation. We see from this that on the days of different response, the animals were in somewhat unlike conditions, which may account for the irregularity of response. The significance of this will be dealt with later. The important matter here is, that the animals, when in the same condition and under the same circumstances, have a fairly constant geotaxis from day to day. One of the most marked cases is that of number 24. This animal was experimented on at different times for a period of three weeks. During this period, it was always active and in good condition, and, as the table shows, at all times, exhibited nearly the same percentage negative geotaxis. At the last trial made, it responded irregularly, and so slowly, — at one time not changing its position for thirty minutes, — that I had to give up the attempt to obtain a series. This was often the case with other individuals after a few definite responses.

Tests were then made on the geotaxis of the same individuals at different times of the same day. Considering the slug's normal environment, it would not be surprising if, for instance, it should show an upward tendency in the evening and a downward geotaxis in the daytime. Its nocturnal habits and dislike of daylight might give it a different geotactic instinct at night from that of the daytime. I insert here a table (III.) giving the results of a few experiments bearing on this point. As the table shows, the response is pretty constant at different times of the same day. The one exception is number 2. That it was negative on one evening at 7 P. M., may be explained by the fact that its condition was not good. Moreover, on another evening at the same time the animal had become positively geotactic.

TABLE III.

GEOTAXIS OF THREE INDIVIDUALS AT DIFFERENT TIMES IN THE DAY.

Animal No.	Time of Day.	Number of Trials.		% Geotaxis.	Condition of Mucus.
		+	–		
1	8.00 A.M.	5	2	+71.4	Good
	1.30 P.M.	6	3	+66.6	Rather Dry
	8.30 P.M.	7	4	+63.6	Tail Dry
2	1.30 P.M.	16	1	+94.1	Good
	7.00 P.M.	9	18	–66.6	Rather Dry
	7.00 P.M.	12	5	+70.5	Fair
	10.00 P.M.	6	3	+66.6	Fair
3	7.00 A.M.	3	8	–72.7	Fair
	1.30 P.M.	0	5	–100.	Fair

From the observations recorded in Tables III. and IV., it is plain that the geotactic response is not due to purely accidental factors, but can be explained only by some marked difference between the individual animals. The first thought is that differences in response are due to a difference in size, and the facts seem to give some support to that explanation. Most of the positively geotactic individuals were found among the small and medium-sized animals, and nearly all the negative animals were of large size. Moreover, the few indifferent individuals were of medium size. This, however, was not an invariable rule. Small animals were sometimes negatively geotactic and, occasionally, a large slug would migrate earthward.

A second, clearly important, factor is the condition of the animal's mucus. As shown by the preceding experiments, animals, positively geotactic when normal, became negatively geotactic when lacking in an abundance of sticky slime; *e. g.* animal 2, Table III., and animals 2 and 7, Table IV. On the other hand, in one instance (22 b), a negative animal, when extremely sticky, went downwards. Abundant, sticky mucus is evidently connected with a downward migration, and dryness seems to force the animal to take an upward direction. But these facts are not enough to explain all responses. For sometimes two animals of nearly the same size and in equally good condition gave different geotactic responses. We must look for other differences. It will, however, be necessary first to refer briefly to the form and external appearance of the slug.

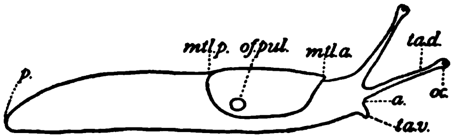

FIGURE 1.

Outline of Limax maximus. *mtl. a.*, anterior edge of mantle; *mtl. p.*, posterior edge of mantle; *a.* to *mtl. p.*, anterior region of body; *mtl. p.* to *p.*, posterior region; *oc.*, eye; *ta. d.*, dorsal tentacle; *ta. v.*, ventral tentacle; *of. pul.*, pulmonary orifice.

The slug, if we except the respiratory opening on the right side of the body, is externally bilaterally symmetrical. It has no external shell. There are two pairs of tentacles, — a dorsal pair bearing the eyes and a smaller ventral pair. The mantle extends from the neck, ventrally, to near the edge of the foot. Posteriorly, it forms a prominent fold, as indicated in the figure, which may be used to separate the body into an anterior and a posterior region. Observations of the animal reveal that it has very different degrees of control over these two regions of the body. In locomotion, the head end of the body, back as far as the respiratory opening, is freely swung about from side to side and determines the axis of orientation of the animal. Over the posterior region, the animal seems ordinarily to have very imperfect control. The relation between the two regions is crudely that of a span of horses to a chain of wagons which they are pulling. When the horses change direction, the wagons come only slowly around into position one after the other, and there is likely to be some slipping in the process, especially if it takes place on a down grade. In watching the slug, I saw that the adhesion of the anterior region appeared considerably greater than that of the posterior. When the animal gets dry, it does so first at the posterior region. The tip of the tail is the part first to lose its clinging power, and it may curl up dorsally as a result of the drying process. If an animal which is thus beginning to deteriorate in its supply of mucus be put on a glass plate and the plate raised into a vertical position, the slug will move along and desperately cling to the plate with the anterior part of its body. The posterior region will gradually swing downward as a result of the pull of gravity, and, in consequence, the animal's head will eventually be directed upward. From this, we are justified in concluding that the same principle will operate, although to a considerably less degree, in the animal's normal condition. A hasty examination showed that there was a good deal of variation in the proportions of the two regions in different individuals. As a crude and easy way of estimating these proportions, I measured the length in millimeters of the anterior region from the tip of the head to the posterior fold of the mantle, and similarly the length of the posterior region from that fold to the tip of the tail.*

The results from 27 animals thus measured are given in Table IV.

The individuals (Table IV.) are arranged in a series, beginning with those in which the two regions are most nearly of the same length and ending with those in which the disproportion is greatest. In animal No. 1, the length of the anterior region is 83.3 per cent (column 8) of the posterior; that is, the ratio is almost one to one. In No. 25, the anterior region is only 45 per cent as long as the posterior, or less than half its length.

The fifth column in the table gives the geotaxis of individuals in per

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* The measurements were made when the animal was extended and moving across the plate. The amount of elongation varies a good deal, but the regions retain pretty closely their relative proportions.

TABLE IV.

SUMMARY OF GEOTACTIC RESULTS.

Animal No.	Series of Observations on Different Days.	Number of Trials.		% Geotaxis.	Length of Anterior Region in mm.	Length of Posterior Region in mm.	Ratio of Ant. to Post. Region in per cents.	Condition of Animal.
		+	−					
1	. . .	10	0	+100.	20	24	83.3	Good.
2	a	6	3	+ 66.6	18	22	82.	Fair.
2	b	5	13	− 72.2	18	22	82,	Dry.
2	c	12	5	+ 70.5	18	22	82.	Good.
3	. . .	6	0	+100.	6.5	8	81.	Good.
4	. . .	6	1	+ 85.7	17	21	81.	Good.
5	. . .	5	2	+ 71.4	24	30	80.	Good.
6	. . .	7	1	+ 87.5	11	15	78.	Good.
7	a	0	4	−100.	26	40	65.	?
7	b	9	6	+ 60.	26	40	65.	Rather dry.
7	c	7	3	+ 70.	26	40	65.	Slow.
7	d	0	2	−100.	26	40	65.	Tail slips.
8	a	12	6	+ 66.6	?	?	?	Good.
8	b	17	1	+ 94.4	?	?	?	Good.
9	. . .	0	6	−100.	21	33	63.6	Tail slips.
10	. . .	2	8	− 80.	20	32	62.	Mucus watery.
11	. . .	14	3	+ 82.3	21	34	61.8	Good.
12	. . .	6	0	+100.	27	44	61.	Good.
13	a	5	12	− 70.5	17	25	61.	Active.
13	b	3	8	− 72.7	17	25	61.	Active.
14	. . .	9	3	+ 75.	20	33	60.5	Good.
15	. . .	1	8	− 88.8	24	40	60.	Fair.
16	. . .	0	10	−100.	28	50	56.	Good.
17	. . .	6	14	− 70.	30	55	54.5	Good.
18	. . .	8	3	+ 72.7	30	56	53.5	Extrem'ly sticky.
19	. . .	0	8	−100.	23	43	53.5	Fair.
20	. . .	1	5	−83.3	17	32	53.	Good.
21	. . .	4	12	− 75.	21	40	52.5	Good.
22	a	7	8	− 53.3	41	79	52.	Sticky.
22	b	8	5	+ 61.5	41	79	52.	Very sticky.
23	a	5	9	− 64.2	18	36	50.	Good.
23	b	6	10	− 62.5	18	36	50.	Good.
23	c	0	5	−100.	18	36	50.	Good.
23	d	0	4	−100.	18	36	50.	Good.
24	a	3	15	− 83.3	27	54	50.	Good.
24	b	3	14	− 82.3	27	54	50.	Good.
24	c	2	19	− 90.5	27	54	50.	Good.
24	d	4	19	− 82.6	27	54	50.	Good.
24	e	3	14	− 82.3	27	54	50.	Good.
25	a	7	17	− 70.8	21	44	48.8	Good.
25	b	4	14	− 77.7	21	44	48.8	Good.
25	c	0	12	−100.	21	44	48.8	Dry.
26	. . .	3	15	− 83.3	?	?	45.	Good.
27	a	0	14	−100.	32	71	45.	Good.
27	b	1	5	− 83.3	32	71	45.	Good.

cents. The table includes those animals which were fairly active in response but does not give individuals obviously unable to respond because of a lack of slime secretion. The positively geotactic animals, with two exceptions, are all found in the upper half of the table and almost all the negative animals in the lower half. Supposing other conditions the same, we can say that those animals in which the ratio of anterior to posterior regions is as 2 : 3, or greater, will be positively geotactic. Those between the ratios of 2 : 3 and 3 : 5 will be more uncertain in their geotaxis, which will depend largely on the combination of other conditions. Finally, those in which the ratio is less than 3 : 5 will almost invariably be negatively geotactic. The nearer one gets to the extremes, the greater the accuracy of prediction. This prediction, it is understood, applies only to animals tested on the glass plate.

An examination of the ninth column shows that the few cases of negative geotaxis occurring in the positive half of the table are probably due to a deficiency in the second most important factor affecting the geotaxis; namely, the condition of the slime secretion of the animal. This secretion may be deficient either (1) in quantity, as in the case of slug 2 b; or (2) in quality, as was the case with slug 10. Of the two cases of positive geotaxis occurring in the negative half of the table, the first, that of slug 18, is easily explained as due to an extraordinary tenacity of the mucus. Moreover in this, and more markedly in the case of slug 22 b, the slugs were very large and rather slow in their movements. Slug 22 b, instead of moving ahead actively, like most slugs when in good condition, often swung its head toward the earth without any foreward movement, and hence did not give the pull of gravity the most favorable opportunity to work on the posterior region of its body. This connects itself with a general observation on all the animals. When active, they are usually very precise and uniform in their responses. If stupid, slow, and averse to movement, — a condition in which the best of them sometimes get, — they will either obstinately refuse to move, or else, keeping the posterior region firmly fixed, will swing the head end toward the earth. Sometimes such a slug will slowly move in a circle, first down then up, and finally curl itself up, like a dog by the fireplace, and apparently go to sleep. This peculiarity may be connected with the food conditions of the animals, as will be shown in a set of experiments to be given later on.

The two most important factors in determining the geotaxis of individual slugs are, therefore: first, the proportion of the anterior (mantle-covered) and posterior (uncovered) regions of the body; secondly, the character of the slime secretion of the animal. If accurate measurements were made of the two regions of the body, we might obtain exactly the relative weights of these two factors. By means of a spring balance, the effectiveness of the mucus in counteracting gravity could be ascertained with a fair degree of accuracy. A large number of such observations in connection with geotactic tests might, finally, enable us to state precisely what combination of the two factors — weight of regions and strength of mucus — would be necessary to make an animal positively or negatively geotactic. I have made no such calculations, and it would perhaps not be worth the trouble. The suggestion is instructive, however, as indicating the possibilities of predicting, with a certain degree of exactness, a phenomenon which seems at first sight to be entirely haphazard. Perhaps perfect mathematical exactness would, however, never be possible in this case, for, as I shall show a little later, other factors of importance probably enter in to modify the results. However, these too are not out of the reach of precise definition.

Certain slugs are negatively geotactic because gravity pulls the posterior region of the body down faster than it does the anterior region. Since in all slugs the posterior region somewhat exceeds in length the anterior, we should expect all animals to respond in the same way, provided gravity acted in only a mechanical way. But about the same number of slugs go down as go up. Therefore, there must be some other factor, such as an inherent tendency, impelling these positive slugs to seek the earth. But if so, is it not probable that *all* slugs have this inherent tendency to move towards the earth, the tendency being obscured in the negative slug by the superior force of the mechanical difficulties to be overcome? The fact that positive slugs, when deficient in means of resisting the pull of gravity, — that is, when dry, — assume a negative geotaxis, shows that the inherent tendency *is* sometimes obscured. If this hypothesis is true, then we ought to be able, by diminishing the force of gravity, or better, by increasing the animal's powers of resisting the disproportionate pull on the posterior region, to make the negative animals become positive. Similarly, if this mechanical difficulty of adhesion is the cause of negative geotaxis, we ought, by increasing it, to be able to compel positive animals to become negative. The first end may be attained by substituting for the glass plate a wooden one, which will presumably offer the animal a better chance of adhesion. The second end may be reached by substituting for the glass plate one which has been coated with vaseline or a similar substance. Both ends may also be attained, to a certain extent, by increasing or decreasing the angle of inclination of the plate. An examination of the tables given by Davenport and Perkins ('97, p. 103) shows that the largest average number of negative responses occurred when the glass plate was vertical; that is, when the mechanical difficulties were greatest. There is a gradual decrease in this average (and a corresponding increase in the average number of positive responses), as the angles of inclination of the plate with the horizon were diminished from 90° to 60°, 45°, and 30° successively. At the still smaller inclinations of 22½°, 15°, 7°, and 0° (*i.e.*, horizontal), however, there is on the whole an *increase* in the average number of negative responses, though this is quite irregular. Since the proportion of anterior to posterior region of the animals experimented on is not known, we cannot tell how far this factor may have been the cause of this irregularity in the sense of the response.

I have made a few experiments by varying for the same individual the angle of inclination of the plate. The animals were all in good condition throughout the experiments. The results — given in Table V. — show a decided increase in negative geotaxis with increase in the angle of inclination.

TABLE V.

Per Cent of Geotaxis at Different Angles of Inclination of the Support.

Animal No.	Angle of Inclination.	Number of Trials.		% Geotaxis.	Condition of Animal.
		+	−		
1	45°	8	2	+ 80.	Good.
1	90°	0	14	−100.	Good.
2	45°	7	1	+ 87.5	Good.
2	70°	8	8	+ 72.7	Good.
8	45°	2	8	− 80.	Good.
8	90°	0	10	−100.	Good.

The most striking case is the complete reversal of geotaxis, seen in the first animal experimented on.

Still more conclusive results were obtained by the substitution of wood or vaselined glass surfaces for the clean glass plate. In order to make sure that the animal's power to hold on varied with different surfaces, and to determine approximately the relative strength of the adhesion, Dr. Davenport suggested the use of a delicate spring balance, such as are used in weighing letters. The animal was placed on a horizontal glass plate. When it had oriented itself, and was moving forward, the pan of a letter balance was held against the side of the animal and gradually increased pressure exerted until the animal was made to slip along the plate. The maximum reading (in ounces) on the indicator was noted. Then the same animal was placed on a wooden plate and a similar test made under like conditions of movement and activity. The same was done on the vaselined plate. A number of such tests were made on each individual. In order to avoid possible differences in results due to a gradual deterioration in the condition of the animal, the sequence of the surfaces was varied in the successive sets (three) of trials so that each surface was once employed for the first experiment of a set. This method proved fairly satisfactory and gave in some instances very striking results.

TABLE VI.

Amount of Pressure required to dislodge the Slug from Different Horizontal Surfaces.

Animal No.	Wood.	Glass.	Vaselined Glass.
1	1.8 ounces	1.5 ounces	.23 ounces
2	1.25 ounces	.67 ounces	.34 ounces
3	3.16 ounces	2.16 ounces	1.55 ounces
4	4.33 ounces	2.55 ounces	1.55 ounces
5	3. ounces	1.16 ounces	.50 ounces
6	5.7 ounces	3.50 ounces	1.52 ounces

The results recorded for each individual are the averages of three trials on each of the surfaces used. The table shows a considerable difference in the degree of adhesion to the different surfaces. In the last four cases the animals were all very large. They were in excellent condition, having just been captured, and secreted a sticky slime in large quantities.

After being on the vaselined surface, there was a noticeable decrease in the power to hold on to the glass or wood, due probably to the vaseline which still adhered to the animal. Regarding these cases as typical of all slugs, we can say that the wooden surface affords a condition nearly twice as favorable as that of the glass plate for the exhibition of an internal tendency. The vaselined surface, on the contrary, is only about half as favorable as the glass plate; that is, it doubles the obstacles. As a general rule, owing to the irregularities of other influences, the difference between the different surfaces would be, probably, somewhat less. For active, well-conditioned animals, however, we have no hesitation in concluding that the ratios obtained from these cases are fairly representative.

Having thus established the fact that the character of the surface does modify the animal's power to attach itself, I next give a table (VII.) showing the results of a series of experiments on twelve different individ-

TABLE VII.

Geotaxis of the Slug on Different Surfaces.

Animal No.	Ratio of Anterior to Posterior Parts in %.	Plate at Inclination of 45°.								
		Wooden Plate.			Glass Plate.			Vaselined Glass Plate.		
		No. of Trials.		% Geotaxis.	No. of Trials.		% Geotaxis.	No. of Trials.		% Geotaxis.
		+	—		+	—		+	—	
1	61.	9	8	+ 53.	0	5	—100.	0	5	—100.
2	?	5	0	+100.	6	5	+ 54.5	0	9	—100.
3	52.	5	0	+100.	1	3	— 75.	0	5	—100.
4	47.	7	0	+100.	1	8	— 88.8	0	slips	0
5	76.	5	2	+ 71.4	5	1	+ 83.3	1	3	— 75.
6	50.	7	3	+ 70.	2	8	— 80.	0	slips	0
7	66.6	9	1	+ 90.	1	9	— 90.	0	slips	0
8	83.3	5	0	+100.	10	0	+100.	4	6	— 60.
9	56.	9	1	+ 90.	0	10	—100.	0	0	0
10	61.	5	1	+ 83.3	5	1	+ 83.3	1	5	— 83.3
11	53.	8	0	+100.	1	5	— 83.3	0	slips	0
12	53.5	6	0	+100.	7	2	+ 77.7	2	slips	0

uals. The geotaxis of each animal was tested on three different surfaces, — the glass plate, a circular wooden plate, and a glass plate coated with vaseline. Care was taken to have other conditions as nearly as possible the same. Circular plates were employed so that the animal could be rotated into a horizontal position without being touched by the hand. In several cases a series was made on an animal using the glass surface; the animal was then transferred to a wooden plate and the same number of trials made; the same individual was then put back on the glass plate and as many more tests were made; finally, it was returned to the wooden plate and an equal number of observations made. The same thing was tried alternating between glass and vaselined surfaces.

The second column shows what per cent of the length of the posterior region of the animal's body its anterior region is, as previously defined. A comparison of the columns "% Geotaxis" under the different conditions at once shows, in nearly every case, a marked difference in the geotactic response with the three kinds of surfaces. The same number of trials was not always made on a given animal under the different conditions, so that the comparisons are not always on exactly the same basis. The results, however, prove pretty conclusively that all animals have an inherent tendency to move toward the earth. On the glass plate, the animals moving upward and downward are about equal in number, the reasons for which we have already given. On the wooden plate, which affords the best of the three surfaces for adhesion, all the animals have become positive. A vaselined surface offers still greater difficulties to positively geotactic responses; it compels the positively geotactic animals to become negative (Nos. 2, 5, 8, 10). Some animals are utterly unable to adjust themselves to this extraordinary condition, especially if not endowed with the power of secreting excellent mucus. These animals either vainly cling with the anterior end of the body to the plate, while the posterior region slips downward, thus directing the animal up, or they roll off the plate altogether as soon as it is placed in an inclined position. For this reason some of the animals negatively geotactic on the glass plate gave no geotactic response when they were placed on the vaselined surface. These facts, then, conclusively answer in the affirmative our second question. All slugs have a tendency to move toward the earth.

Now the question naturally comes up, Can we not assist this tendency in those animals which are negatively geotactic on a glass surface by bringing some other stimulus — light, for example — to bear upon them? This slug is negatively phototactic to strong light, as the third part of this investigation will show. By exposing the animals to strong light, can we not make the desire for darkness coöperate with the inherent positive geotactic tendency to such an extent that the two together will overcome all mechanical difficulties and cause the animal to move downward? The following table (VIII.) answers this question in the affirmative.

TABLE VIII.

GEOTAXIS OF SLUG ON GLASS PLATE AT AN ANGLE OF 45° INFLUENCED (1) BY GRAVITY ALONE, AND (2) BY GRAVITY AND STRONG LIGHT.

Animal No.	Size.	Gravity alone.			Gravity + Influence of Strong Light.		
		No. of Trials.		% Geotaxis.	No. of Trials.		% Geotaxis.
		+	−		+	−	
1	Big	0	17	−100.	8	8	+ 50.
2	Big	0	14	−100.	7	5	+ 58.3
3	Big	1	7	− 87.5	2	2	± 50.
4	Big	0	5	−100.	2	4	+ 66 6
5	Medium	3	9	− 75.	4	4	± 50.
6	Small	0	5	−100.	0	5	−100.

These experiments were carried on in the evening. The animal was first tested on a glass plate at an angle of 45° in the dark, in the ordinary way. Then it was placed on a horizontal glass plate and strong lamp light thrown directly upon it for a few seconds. In most cases it immediately gave a negative response to the light. When definitely oriented, the plate was again placed in the box at an angle of 45° and the box covered with a black cloth. Two or three geotactic observations were then taken, and the animal again exposed to strong light. The exposure to light was repeated about three times in the course of ten observations. The table shows that the influence of light has been to change a condition of strong negative geotaxis to one of indifference. The only exception is No. 6, which seemed little affected by the light. I hope to make a fuller study of the combined action of light and gravity later.

It has been said that all slugs have an innate tendency to move toward the earth. Now, this tendency is probably due to the environment and habits of the animal. The slug, we know, is nocturnal in its habits. In the nighttime, it is actively moving about in search of food. In the daytime, it is inactive and seeks concealment, which is of course accomplished by moving toward the earth. In hunting for food, it must naturally do some climbing. These facts lead us to expect a possible difference between the geotactic response of the nighttime and that of the daytime. My experiments in this matter, however, gave inconclusive results. But the animals experimented on were not in their normal environment. There was no light and little change in temperature to assist the instinct, if it exists, in divining night from day. Moreover they did not have to seek food, for it was constantly supplied them. Such being the case, the instinct of concealment would be the main environmental influence on the animal, and this impels it toward the earth.

These experiments have shown, then, that when the mechanical conditions are favorable, most animals exhibit a positive geotaxis. This is as we should expect. There were, however, a few exceptions. A few animals went up when all the factors enumerated seemed to point to the probability of a downward movement, and there were also a few animals which went down when the mechanical difficulties were such as should have impelled them upward. As previously noted, the upward-moving animals sometimes displayed an unusual amount of activity, and the exceptional cases of positive geotaxis in the negative group were those of animals usually slow and stupid. As the effort was constantly made to select only fairly active animals in good condition for producing mucus, there were not many of these exceptions. Knowing the habits of the animal, we may naturally associate its activity with its food condition.

The question then comes up, Does the state of the animal's nutrition affect its tendency to move toward the earth? Does a poorly nourished animal respond to the stimulus of gravity differently from a well-nourished individual? To get an answer to this question, four animals were put into a small box which contained nothing but moist earth. The slugs were kept there for three days, and a series of geotactic tests was then made upon them. Two of the four individuals were inactive, and so unsatisfactory in response that no series was obtained. The other two were rather restless, but precise in response. All the animals were then returned to the box and supplied with fresh cabbage leaves. The next morning another series of geotactic stimuli was given. The rather meagre results given in Table IX. are perhaps not worth very much, since only one individual (No. 1) out of the four responded well in both cases.

The ratios given in the second column (Table IX)indicate that slugs Nos. 1 and 2 belong with those of the positive half of Col. 8, Table IV. I unfortunately neglected to control these experiments by observing the geotaxis *before* the animals were deprived of food. In

TABLE IX.

COMPARISON OF RESPONSES OF INDIVIDUALS WHEN POORLY NOURISHED AND WHEN WELL NOURISHED.

Animal No.	Proportionate Ratio of Anterior to Posterior Region.	Poorly nourished.			Well nourished.		
		No. of Trials.		% Geotaxis.	No. of Trials.		% Geotaxis.
		+	−		+	−	
1	70.	3	9	−75.	7	2	+77.7
2	60.	1	8	−88.8	4	1	+80.
3	56.	0	0	0	12	6	+66.6

both instances (Nos. 1 and 2) the animals were rather dry, and they were not noticeably different in this respect after being well fed. No. 2 was less active and less precise in response after it had had plenty of food. I think these experiments too few to warrant laying much stress upon them, but I have given them here because they at least point in the direction of what we might reasonably expect, since the natural desire of the animal to escape from its narrow prison and the impulse to seek food would both tend to make it go up, if given the opportunity.

Another element which may alter the slug's inherent geotaxis is probably the state of fear. This element may be combined with the impulse to seek food, as is perhaps the case in the instances just given, or it may operate by itself. Animals which had just been captured were always kept in a small tin box. The captured animals would thrust themselves between the box and lid, which was not perfectly tight, in their endeavors to escape, and they had to be frequently pushed back. When they were transferred to the large box mentioned at page 187, it was always found that they had all collected in the upper part of the smaller box. This may have been solely for the purpose of getting air, but such animals put on a glass plate were exceedingly active and restless, and usually exhibited a decided negative geotaxis. I have not made any careful set of experiments to find out whether these negatively geotactic animals afterwards became positive. In one instance, I confined over night in a small flower-jar a slug (not a freshly captured one) which had shown a very decided positive geotaxis. In the morning it was found at the top of the jar, and, when placed on a glass plate, showed great activity, as though it sought to escape. In every one of the tests which I then made, it responded negatively. From these few observations, it would seem that fear, by impelling the animal to escape from captivity, may alter its geotatic response. Such freshly captured slugs, moreover, which seem unusually restless and excited, respond more capriciously to the stimulation of light, as some later experiments will show.

SUMMARY OF PART II.

The results of the foregoing experiments warrant the following conclusions: —

1. On an inclined *glass plate*, all slugs give a geotactic response.
2. Certain slugs give a decided positive, others a markedly negative geotactic response; a few are somewhat indifferent.
3. The geotaxis of animals kept in confinement does not vary much on different days, nor at different times on the same day.
4. The occasional vagaries in the responses of individual animals are to some extent due to thigmotactic and phototactic influences.
5. The different geotactic response, on a *glass plate*, of different individuals is due mainly to two factors: (*a*) The quantity and quality of the slime secreted, which is a very important factor; (*b*) the relative proportions of the length of the anterior and the posterior regions of the animal's body. All the conditions being the same, it is this factor which "determines whether the head end will be directed up or down."
6. If the ratio of length of anterior to posterior region of body is 2 : 3, or more, and the mucus is of good quality and sufficient quantity, the slug will be positively geotactic.
7. If the ratio is 3 : 5, or less, the animal will usually migrate upward, and the nearer the ratio approaches 1 : 2 the more apt is the slug to respond negatively.
8. In a small number of individuals, in which the ratio lies between 2 : 3 and 3 : 5, the response will depend largely on the condition of the mucus and coöperation of other factors.
9. All slugs have a natural tendency to move towards the earth. This tendency is masked in the animals which are negatively geotactic on a *glass plate* by the greater pull of gravity on the disproportionately larger and heavier posterior region of the animal.
10. The general downward tendency may vary normally at different times of the day, owing to the animal's habit of remaining in concealment in the daytime and feeding at night.

III. PHOTOTAXIS.

The influence of light on the direction of locomotion has been very generally noticed among organisms, even the mostly widely separated. The swarm spores of many algae, desmids, and other lowly organized plants, are as truly responsive to light stimuli as are crustaceans or vertebrates. According to the character and direction of the stimulating light rays, two kinds of light responses have been distinguished. Phototaxis is the response with reference to the *direction* of the rays of light. The organism moves in the path of the ray, either positively (toward) or negatively (away from it). The response to different *intensities* of light from which the directive force of the rays has been eliminated is known as photopathy. A photopathic animal is one that selects, out of a series of uniformly increasing intensities of light, a limited field of a certain intensity.

Some animals, like butterflies and fresh-water Entomostraca, are strikingly positively phototactic to diffuse daylight; others, such as the earthworm and the leech, are as pronouncedly negative. The kind of response (positive or negative) may be different in closely allied forms and in different stages of development of the same species. For example, butterflies are attracted by strong sunlight, while moths are repelled by it. The adult house fly is positively phototactic to daylight; its larva, negatively (Loeb, '90, pp. 69–77, 81–83).

The phototactic sense has been shown in some forms to change with different intensities of light. Thus, Famintzin ('67) found that swarm spores positively phototactic to a certain intensity of light became negative to a light of greater intensity. The same phenomenon has been found true of various flagellates, desmids, diatoms, oscillariae, etc. Wilson ('91, p. 414) found that Hydra fusca was attracted by diffuse daylight and repelled by strong sunlight. Finally, the moth's liking for candlelight and aversion to daylight is well known. The fact that many organisms are photopathic — that is, have a preference for light of a certain intensity — makes it probable, in connection with these observed variations in phototactic responses, that, for most organisms, there is an optimum intensity to which they will respond positively. This optimum will vary widely in different species, probably according to the habits and the usual environment of the species. Inhabitants of sunny pools or the open air will have an optimum of relatively high intensity; those which dwell in the ground or in shady places will have a correspondingly lower optimum. May it not be that every organism will respond positively to a certain range of light intensities and negatively to another range of intensities which is greater? The nature of the phototaxis may sometimes be gradually changed by organisms becoming acclimated to new conditions. Verworn ('89, pp. 47–49) found that a culture of the diatom Navicula brevis, which ordinarily is negatively phototactic to very weak light, became positively phototactic when reared for several weeks near a window. Groom und Loeb ('90, pp. 166–167) found that young Nauplius larvae of Balanus which were at first positively phototactic to daylight became negatively phototactic later in the day, probably as the result of the accumulated effects of this exposure.

The character of the light responses, as was the case with geotaxis, depends also to a certain extent on other external conditions, such as those of temperature, the states of density and pressure, and various chemical influences. Polygordius larvae, when gradually cooled from 16.5° C. to 6° C., were found by Loeb ('93, pp. 90–96), to change from a negative to a positive phototaxis. Like results were obtained by him from Copepoda. When the temperature was raised from 6° C. to 16° C., the animals again became negative. Increasing the density of sea-water by the addition of sodium chloride produced a change from a negative to a positive response, thus acting like diminished temperature. Engelmann ('82, pp. 391–392) showed the apparent phototactic response of chlorophyllaceous ciliates to be really a chemotactic attraction for oxygen, which chlorophyll can produce only in the light. These facts make it important in any study of light response to consider other possible influences, and above all to take account of the strength of the stimuli used.

Davenport and Perkins ('97) found that the slug (Limax maximus) responded with marked precision to the varying stimuli of gravity at different angles of inclination of the glass plate. The precision of response varied correlatively with the force of gravity. In fact, the parallelism was almost perfect. The question naturally rises, Is there a similar parallelism between other stimuli and their responses?

A very little experimentation shows that the slug is extremely sensitive to light. We have already seen how light may enter in to modify the action of gravity. Casual observation shows that the response is in most cases negative, — the animal moves away from the source of light. Owing to its method of locomotion, the slug is easily experimented on. It moves slowly and deliberately. In regard to its responses to light, the following questions suggest themselves: (1) Are all individuals negatively phototactic to artificial light? (2) Does the precision of response vary correlatively with the intensity? (3) Within what limits of intensity is the animal responsive? (4) Does the kind of response vary at different intensities? (5) Is there a difference in the sensitiveness to light of the two sides of the animal's body? (6) In what part, or parts, of the animal's body does the sensitiveness reside? (7) How does the animal move when in the dark and deprived of all stimulating influences? These various problems came up gradually as the work progressed and were considered in turn. Other interesting studies have suggested themselves in the course of the investigation, but there has not been time to go much beyond a consideration of the questions above proposed. The experiments performed were all phototactic; that is, they were studies of the response of the slug to the direct rays of light.

Methods. — The methods used were simple. For light, the standard candle and the ordinary small Christmas candle, of a one fourth candle power, were employed. The candle was placed in a box 50 cm. (20 inches) high and having a bottom 12.5 cm. (5 inches) wide and 20 cm. (8 inches) long. It could be raised or lowered to any desired position by means of an adjustable stage inside the box. A circular opening in the middle of one of the broad sides of the box 2 cm. (⅞ inches) in diameter permitted the light to pass out. This opening was covered by a piece of oiled paper, so as to give a well-defined uniform source of light. During the experiment the box was closed by a lid. The intensity of the light was varied by altering the distance between the box and the animal. Additional thicknesses of paraffined paper were also employed when it was desired to greatly diminish the intensity of the light. The animal was put on a circular glass plate which rested horizontally on a support, and the box was raised so that the centre of the light opening was in the same horizontal plane as the body of the animal. The movement of the slug from its original position was measured in degrees in the following manner. A circle of the same size as the glass plate was described on a sheet of thin paper and divided by radii into 72 sectors of 5° each. This sheet was pasted to the under side of a second circular glass plate (of the same size as the first), on which also a heavy base line was drawn, corresponding with a diameter of the circle. This second plate was so placed that the centre of the source of light was on a line perpendicular to the base line at its middle point. The slug was put on the first glass plate, which could be rotated so as to bring the animal into any desired position with reference to the base line. The experiments were carried on in a dark room provided at one end with a hinged window which could be easily and quickly thrown open. The window was covered with a thick, black cloth, so that, when closed, external light was almost completely shut off. Unfortunately, it was impossible, owing to the position and nature of the room used, entirely to equalize all conditions. The temperature was not the same from day to day and varied somewhat in different parts of the room. Generally, it was so hot and close that it was necessary to leave an opening between the sashes, and this of course created a slight draft and produced irregularities of temperature. No account was taken of the varying humidity of the atmosphere, a factor which may have somewhat influenced the animal's locomotion. Moreover, as the room was not perfectly light-tight, there were feeble light stimuli in addition to the artificial ones used. However, all these imperfections were but slight, and, since they entered more or less into all the experiments, could not greatly alter the relation between the results, which was the main thing sought in the investigation. Other unestimated possible influences were the nutrition of the animal and such slight thigmotactic stimuli as could not well be avoided.

The strength of the different intensities of light used was measured by moving a piece of paper, the centre of which was oiled, between a light of known intensity and the light whose intensity it was desired to know, until the oiled spot on the paper was not distinguishable from the rest of the paper. The distance from this point to each source of light was then measured. Since the intensity varies inversely as the square of the distances, it is an easy matter to calculate the relative strengths. This method is accurate enough for all ordinary purposes.

Operations and Results. — In beginning any experiment, the slug, as soon as it had definitely oriented itself, was rotated into such a position that the axis of its body coincided with the base line, and its head was at the centre of the disk. The window was then immediately closed and the time noted. At the expiration of 45 seconds, the window was thrown open and the animal's position instantly noted. The extent of positive or negative migration was at first ascertained by finding the length of the arc stretching from the base line to the radius which was *parallel with the axis of the slug's body.* Any movement into the half of the circle toward the source of light was called positive ; any movement into the other half, negative. It would occasionally happen that an animal would at first move into the positive half of the circle and then turn away from the light. In this case the axis of orientation made a negative angle with the base line, although the animal itself lay in the positive half of the circle. Later, in the course of the experiments, the positive or negative movement of the animal was measured by taking the radius which passed *midway between the two tentacles,* without regard to the position of the body axis. A comparison of the two methods showed but little difference in the results. The animals only occasionally made these irregular responses, first in a plus and then a minus direction. As a rule, the migration was unequivocal after the head end had oriented itself to the stimulus. Experiments were made with 18 different intensities of light, each constituting a "series." Six successive observations were made on each individual (3 with the right side exposed; 3 with the left), and from 8 to 14 animals were employed in each "series." *i.e.*, at each intensity of light, making a total of from 48 to 84 observations at each candle power used. A summary of the results for each of 18 such "series" is given in Table X.

The first column gives the number of the series; the second, the intensities of light. This intensity is expressed in terms of the standard candle power at a distance of one meter. The next column (3) shows the total positive migration of the (8 to 14) animals experimented with. Column 4 similarly gives the total negative migration. Column 5 represents the average arithmetical angular deviation from the original position due to phototactic stimuli, effected in a period of 45 seconds by all the slugs, without regard to the positive or negative character of the individual phototaxis. This average was obtained by adding together the average phototactic responses (whether plus or minus) of each *individual* of the series and dividing the result by the number of animals. The average plus or minus phototactic response (algebraic average) for each series (column 6) was obtained by getting the difference between the sums of all the plus and all the minus movements of each *series* and dividing this difference by the number of tests (observations) made. Column 7 gives the number of positively phototactic animals in each series; column 8, the number of negative animals; column 9, the number of indifferent animals; and column 10, the total number of individuals employed in each series. The sequence of the series is not the same as that of the experiments, but is based on gradually diminishing light intensities. I did not determine the possible influence of the heat of the candle for each of the series, but in one series of experiments in the dark (18*b*), a candle, covered (to shut out the light) with an opaque paper of the same thickness as the paraffined paper, was left burning at a distance of 30 cm. (intensity .676 C. P.).

A casual glance at the table at once answers the first of the questions proposed in the statement of the problems (pp. 207–208). All slugs are not negatively phototactic. At the strongest intensity of light used, two animals exhibited a positive phototaxis, — they moved toward the stimu-

TABLE X.

Responses of the Slug to Light.

1	2	3	4	5	6	7	8	9	10
No. of Series.	Intensity of Light.	Total Phototactic Migration in Degrees.		Average Response in Degrees in a Period of 45 Minutes.		No. of Animals.			
		+	−	Arithmetrical Sum.	Algebraic Sum.	+	−	0	Total.
1	.676	330	2155	45.5	−38.	2	6	0	8
2	.382	625	2772	40.	−25.5	2	12	0	14
3	.169	440	2430	27.5	−25.5	2	11	0	13
4	.042,4	625	1330	26.	−11.7	2	8	0	10
5	.010,5	250	1165	17.6	−15.	2	8	0	10
6	.004,7	830	1140	16.1	− 5.1	3	7	0	10
7	.001,69	405	760	9.1	− 6.	3	6	1	10
8	.000,754	695	595	13.	+ 1.4	7	5	0	12
9	.000,424	1145	895	17.	+ 3.5	6	4	2	12
10	.000,260	823	845	14.5	+ 7.9	7	3	0	10
7*a*	.001,69	365	480	4.6	+ 1.7	4	7	0	11
8*a*	.000,754	845	345	11.8	+ 8.3	7	3	0	10
9*a*	.000,424	985	180	14.7	+14.2	9	1	0	10
10*a*	.000,260	740	435	11.	+ 4.2	8	4	0	12
11	.000,022	1395	55	22.3	+22.3	10	0	0	10
12	.000,009,6	630	515	8.6	+ 2.	7	3	0	10
13	.000,003,35	865	255	13.	+10.	8	2	0	10
14	.000,002,00	800	170	10.5	+10.5	9	0	1	10
15	.000,001,26	850	415	11.1	+ 7.2	7	3	0	10
16	.000,000,185	1375	145	24.	+20.5	7	3	0	10
17	.000,000,018,8	445	870	8.9	+ 1.	6	4	0	10
18*a*	Darkness.	1440	1290	3.6	+ 1.2	10	8	2	20
18*b*	" with candle heat.	475	635	8.7	− 3.	3	6	0	10

lating light rays. Here, then, arises another problem, similar to the one treated of in the first part of this paper, viz., What determines whether a particular slug shall be positively or negatively phototactic? In the first series of experiments — in fact throughout this whole set — the animals used were about equally divided between large, small, and medium-sized individuals. The two positive animals in series 1 were both of large size. They were very active. The only peculiarity wherein they seemed to differ from other individuals was in the unusually sticky character of the slime. Whether there is any correlation between this fact and the liking for strong light, I am not prepared to say. It is possible — and certain observations seem to indicate that it is highly probable — that the food conditions of the animals have some influence on their responses to light, as they were shown to have on their responses to gravity. The psychic state of the animal is also to some extent, I think, a factor. Freshly caught slugs when put on a glass plate sometimes acted as if in great fear. They displayed unusual activity and were very erratic in their movements. If forcibly checked or held, they made strenuous efforts to escape. The great activity of the positive individuals indicates a possible state of fear. One animal in particular seemed highly abnormal. Several times it moved directly toward the circular field of light and even placed its tentacles against the oiled paper which covered the opening. This was the only individual in the whole course of the experiments which exhibited a response like that of moths. No definite set of experiments was planned or carried out in regard to this matter.

As we run down column 5, we see that the average arithmetical response varies quite strikingly at the different intensities. The first seven series show a gradual decrease in the average response as the strength of the light is diminished. Although not so regular, there is also a gradual decrease in the degree of negative response on the part of these seven groups of animals, as shown by the average algebraic sums of their responses (column 6).

Owing to the constant dying off and deterioration of the stock, it was found impossible to use the same set of animals in all the different series of experiments. Moreover, this was not desirable, for the reason that an animal which is constantly experimented on gradually loses its sensitiveness, and thus its responses become untrustworthy. Not knowing the factors which determine the kind of phototaxis, it was of course impossible to make a uniform selection in this respect. We see, however, that the number of negative animals (column 8) is less at the weaker intensities than at the stronger. When we come to series 8 of the table, we meet with a new condition of affairs. Instead of a still further decrease in the amount of deviation, there is a sudden slight increase, from 9.1° to 13°, and a reversal in phototaxis for the series from an average response of —6° to + 1.4°. The number of positive individuals has increased from 3 to 7. It was because of this striking change that it was thought best to repeat this series and the three succeeding ones on another set of animals. The absolute positive or negative migration was this time taken without regard to the position of the body axis. Series 7*a*, 8*a*, 9*a*, and 10*a* are hence taken at the same intensities as 7, 8, 9, and 10 respectively. These repeated series indicate as strongly as the first set that an intensity of .001,69 C. P. very nearly marks the lower limit of negative phototaxis in the slug. Somewhere near a candle power of .000,754, lies an intensity which attracts about as many animals as it repels and in about the same degree. That is, the average phototaxis (algebraic sum) is zero. Below this intensity, there is more attraction than repulsion, and hence there is an average increase of migration toward the light. The table shows that the average positive response increases to some extent correlatively with the diminution of the light intensity, up to a certain point. This point, according to the results here obtained, is the intensity of .000,022 C. P., where the average movement toward the light, in a period of 45 seconds, was through an angle of 22.3°. As we go below this intensity, there is again a falling off in the strength of the positive response, which diminishes, however, with a good deal of irregularity until absolute darkness is reached. These facts will become more apparent from the study of their graphic portrayal in the curve here given.

The continuous line represents the curve as plotted from the results of Table X., column 6; the dotted line, the curve of responses as one may assume theoretically it would have been, could all of the conditions other than intensity of light have been equalized. The abscissae here represent the logarithms of the intensities of light + 10. Beginning with darkness on the left end, there is a constant increase of intensity as we move toward the right. The sines of the angles of response are marked off on the ordinates. Remembering that the left represents a region of weak intensity and the right a region of strong light, that all points above the line x x′ are points of positive response and all points below it of negative response, we can understand the significance of the curve. In the region of strong light, the curve lies far below the line x x′, but gradually rises toward and finally crosses it, as the light

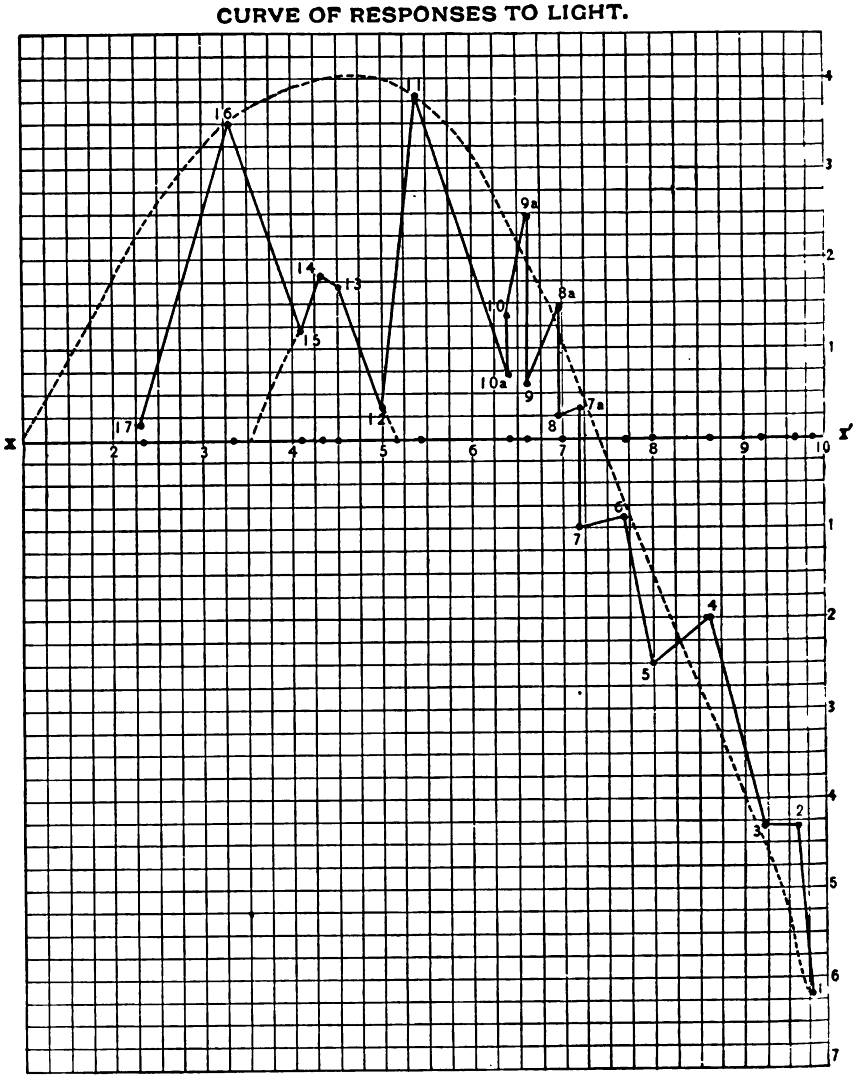


FIGURE 2.

Curve of Responses to Light. *Abscissae* are logarithms of light intensities plus 10; *ordinates* are sines of angles of responses multiplied by 10.

diminishes in strength. Then there is a gradual increase in positive reaction, which reaches its height in a response of +22.°3 at a .000,022 C. P., and then falls toward the zero line as we approach darkness. There is some irregularity in the negative region, but on the whole the rise is gradual. In the region of positive response, there is a considerable lack of regularity, especially marked by the interpolation of one series (12) of very low response between the two series of greatest response. These series intermediate between Nos. 11 and 16 represent later experiments than the two series bearing those numbers. Having obtained such a marked positive response at two widely separated intensities of light, it was thought desirable to get other intermediate series. Hence, the order of the series as arranged in the table, on the basis of gradually diminishing light intensities, does not, as already stated, represent the order in which the series were obtained in my experiments. While the slugs, thus far, had, on the whole, been in good active condition, they were not so in these intermediate series. Although a fresh supply was obtained, all the animals seemed much more stupid and irresponsive than usual. Some of them refused to move, when put on a plate, and many of those that did, responded in a very half-hearted way. The cause of this unusual lack of activity, I could not discover. It may be that a slight change in the food of the animals, which I made at this time, was partly responsible. At any rate, instead of obtaining responses intermediate in amount between those of series 11 and 16 as might have been expected, the results were as have been given. Series 12 was the last one taken. In this, the animals were noticeably more stupid and irresponsive than in any of the preceding experiments. It is very evident from these results, I think, that the precision of response will vary to some slight extent from day to day. The negative responses — those to strong intensities of light — will not be as variable at different times as the positive responses — those to weaker stimuli — as the curve shows. The varying thermal conditions of the room, already mentioned, may have been in part a cause of this irregularity. Furthermore, an animal that has had plenty of food is likely to be stupid and slow in movement and is more apt than a hungry one to seek darkness and concealment. On the other hand, a hungry, active slug will probably exhibit positive phototaxis in a most marked and sometimes abnormal degree, as was the case occasionally with the positive animals at the strongest light intensities. Besides this individual variation, there is, I think, a general variation for all slugs from time to time, for reasons imperfectly known, which will find its expression in curves of different heights.

Thus the less responsive animals of the intermediate but later series mentioned fall into a less prominent curve, as is indicated by the shorter dotted line in the diagram. The curve of positive response approaches, but never actually reaches, the zero line. Even in darkness there is a slight positive migration. This series (No. 18*a*) represents the average of two series of experiments, one of 54 and the other of 66 determinations, each taken at different times during the investigation. This slight positive response — speaking of it as positive with reference to the position of the source of light in the preceding series (17) — may be independent of conditions of light and due to several causes. As mentioned before, the thermal conditions of the room were not uniform, consequently the positive response may have been a response to heat. The movement was away from the window and hence might be explained as a negative response to the repeated inflowing of daylight, when the window was thrown open to make observations. In the last few experiments an opaque screen was put up between the animal and the window. In these cases the average of the responses was slightly negative, so there is some reason to suppose that it was in part the position of the window in the previous experiment that determined the slight positive migration. The actual phototactic responses to the candle light in the positive half of Table X. would then be the observed responses minus this small positive movement in the dark. The actual negative responses to the strong intensities would be the observed responses plus this increment. In series 18*b* the box was placed at a distance of 30 cm. (C. P. 0.676) with the light burning, but the opening was covered with a piece of black paper to shut out the influence of the light while leaving that of heat. The small average response of —3.0 may possibly be regarded as a thermotactic one, and, if so, will have to be deducted from the negatively phototactic response to this intensity of light. For intensities less than the 0.676 C. P., the response to the heat would be correspondingly less.

We can now answer the second and fourth questions (pp. 207–208) by saying, — that the precision of the phototactic response does, on the whole, vary correlatively with the intensity of the light, and that the kind of phototaxis (positive or negative) is not the same for different intensities of light. The slug gives a negative phototactic response to strong light, a positive one to weak intensities, and is neutral to an intensity somewhere between the extremes.

A few individuals were tested successively at different light intensities in order to find out with what precision an individual's phototaxis might vary with a change of intensity.

TABLE XI.

Responses of Individuals to Different Intensities of Light.

Animal No.	Intensity.	Response.	Intensity.	Response.	Intensity.	Response.
1	.382 C. P.	–36.°	.169 C. P.	–34.°	.067 C. P.	–27.°5
2	.382 C. P.	–39.°	.169 C. P.	–14.5°	.067 C. P.	–10.°
3	.382 C. P.	–42.°	.169 C. P.	–31.°	.067 C. P.	–23.°

In all these cases, there is seen to be a gradual diminution in the degree of response as the intensity of light diminishes. Again, from an animal which responded negatively to a certain intensity of light, a positive response could be got by weakening the light sufficiently (Nos. 2 and 3, Table XII.), and a positive animal could be made to give a negative response by using stronger light (No. 1, Table XII.), as the following instances show.

TABLE XII.

Responses of Individuals to Different Intensities of Light.

No.	Intensity.	Response.	Intensity.	Response.	Intensity.	Response.	Intensity.	Response.
1	.382 C. P.	+41.°	Strong Light.	–22.°				
2	.676 C. P.	–15.°	.0424 C.P.	+35.°				
3	.169 C. P.	–37.°	.0188 C.P.	– 2.°5	.0067 C.P.	–32.°	.0047 C.P.	+36.°

No. 3, Table XII., shows a less regular response than any of the other animals. From a response of —37° it drops to one of —2.5°, and, under the influence of a still lower intensity of light, it again rises to a marked negative response of —32.° At a still lower intensity, it gives a striking positive response of +36°. Here, however, we have well illustrated in particular individuals the law laid down for all slugs, — that they are negatively phototactic to strong intensities of light, the precision of response varying correlatively with the intensity of the stimulus; that to weak intensities they are positive; and that to a certain intermediate intensity they are neutral.

A glance at the intensity column (Table X.) shows that the slugs are responsive to a very wide range of intensities. They would probably continue to respond negatively to still stronger light, until the light became strong enough to kill the animal. They respond positively to a light (series 16) less than one three millionth part as intense as the strongest intensity experimented with. The response to the weakest intensity used (series 17) is less than the positive migration in the dark. Hence we cannot speak of this as a phototactic response. This attenuation of light was so weak that I could not be sure I saw it myself, and had constantly to reassure myself by approaching it. The slug is evidently sensitive to a very minute degree of light.

Where does the slug's sensitiveness reside? The first and most natural answer is, that the eyes are the important organs. The matter was tested on five different individuals. The normal phototactic response was first taken with a .676 candle power. Then the dorsal tentacles, bearing the eyes, were snipped off with scissors and the animal again experimented on. The results are given in Table XIII.

TABLE XIII.

EFFECT OF AMPUTATION OF TENTACLES.

Animal No.	Normal Phototactic Response.	Response after Amputation of Dorsal Tentacles.	Ventral Tentacles also Amputated.
1	–70.°	+41.°	
2	–26.°	– 3.°	
3	–44.°	–29.°	+7.°
4	–53.°	+16.°	
5	–65.°	+ 6.°	

As soon as the operation was performed, the stumps were retracted, as the tentacles are when stimulated by touching, or by strong light. After a moment or two, the animal again rolled out the stumps and began moving forward in perfectly normal fashion, as though nothing had happened. The only observable difference was a perhaps slightly increased activity. This table (XIII.) shows a striking effect of the amputation on the phototactic response. In some cases, the animal deviated but very little either positively or negatively from its original position, but kept on moving ahead in a straight line. In other cases, the amputation seemed to cause a change from a strongly negative to a more or less positive response. In the case of animal No. 3, removal of the eyes did not seem to altogether prevent, though it considerably reduced, the negative response. Thereupon, the ventral tentacles were also amputated and the result then was a slight positive response. Since there is probably some shock to the nervous system by the amputation, these results ought to be corroborated by other experiments where the eyes are covered over with some substance to shut off the rays of light. This, I have not yet succeeded in doing satisfactorily.

The experiment of removing only one of the ocular tentacles was tried on two different animals with the following interesting results.

TABLE XIV.

Comparison of Effect of Amputation of Right and Left Dorsal Tentacles.

Animal No.	Normal Phototactic Response.	Response after Amputation of	
		Right Tentacle.	Left Tentacle.
1	−70.°	−27.°	
2	−55.°		+8.°

In the case where the right tentacle was removed, the animal still responded negatively with considerable precision. Amputation of the left tentacle, in the case of No. 2, on the other hand, resulted in a slight positive phototaxis. While these two cases by themselves have little, if any, significance, taken in connection with facts now to be discussed, they seem to indicate a greater degree of sensitiveness to strong light on the part of the left side of the animal's body than the right.

It will be remembered that our thigmotactic experiments pointed to a possible asymmetry in the sensitiveness of the right and left tentacles of the slug. Do we find a similar asymmetry in the responses to light? Table XV. gives the responses of right and left sides respectively for the 18 series. Column 1 gives the number of the series, column 2 the intensities of light, columns 3 and 4 the total angular migrations in a positive and negative direction for the series when the right side was exposed to the light, and the fifth column the algebraic average (positive or negative) phototactic response of the right side. Similarly, the next three columns, 6, 7, and 8, give the responses of the left side. Column 9 represents the total movement of the series in degrees to the right. This result was obtained by adding the total positive responses of the right side (column 3)

TABLE XV.

Comparison of Responses of Right and Left Sides to Light.

1	2	3	4	5	6	7	8	9	10	11
No. of Series.	Intensity of Light.	Responses of Right Side in Degrees.			Responses of Left Side in Degrees.			Total Movement in Degrees to		Total No. of Animals.
		+	−	Average Phototaxis + or −	+	−	Average Phototaxis + or −	Right.	Left.	
1	.676	305	770	−19.	25	1385	−57.	1690	795	8
2	.382	250	1056	−19.	375	1716	−82.	1966	1431	14
3	.169	425	595	− 4.3	15	1835	−46.8	2260	610	13
4	.042,4	255	355	− 3.3	370	975	−20.	1230	725	10
5	.010,5	65	730	−22.	185	435	− 8.	500	915	10
6	.004,7	295	805	−17.	535	335	+ 6.7	630	1340	10
7	.001,69	250	500	− 8.3	155	260	− 3.9	510	655	10
8	.000,754	280	330	− 1.4	415	265	+ 4.1	545	745	12
9	.000,424	530	645	− 3.	615	250	−10.	780	1260	12
10	.000,260	435	245	+ 6.3	388	100	+ 9.6	535	633	10
7*a*	.001,69	250	210	+ 1.2	115	270	− 5.	520	325	11
8*a*	000,754	410	165	+ 8.1	435	180	+ 8.5	590	600	10
9*a*	.000,424	380	75	+10.	605	55	+17.	435	680	10
10*a*	.000,260	560	230	+ 9.	180	205	− 0.7	765	410	12
11	.000,022	955	0	+31.7	440	55	+12.7	1010	440	10
12	.000,009,6	160	275	− 3.8	470	240	+ 7.6	400	745	10
13	.000,003,35	460	120	+11.7	405	185	+ 9.	595	525	10
14	.000,002,0	410	90	+10.7	390	80	+10.3	490	480	10
15	.000,001,26	395	320	+ 2.5	455	95	+12.	490	775	10
16	.000,000,185	915	40	+29.	460	105	+12.	1020	500	10
17	.000,000,018,8	210	215	− 0.2	235	155	+ 2.6	365	450	10
18*a*	Darkness	1220	240	+16.	220	1050	−13.8	2270	460	20
18*b*	“ with candle heat	155	495	−10.	320	140	+ 7.	295	815	10
Totals		9570	8506		7808	10321		19891	16314	
“ less 18*a* & 18*b*		8195	7771		7268	9131		17326	15039	

and the total negative responses of the left side (column 7), — these responses being necessarily right-hand movements. The total movement in degrees to the left (column 10) was likewise obtained by adding the total negative responses of the right side and the positive responses of the left side. Column 11 gives the total number of animals used in each series.

In the region of negative phototaxis, the total positive and negative angular migrations, and the average negative phototaxis of all the series (1–7, inclusive) when the right and left sides respectively were turned toward the light, were as follows.

TABLE XVI.

SUM OF THE RESPONSES OF RIGHT AND LEFT SIDES WHEN PHOTOTAXIS IS NEGATIVE.

Side turned toward Light.	Total Angular Migration.		Average Negative Phototaxis.
	+	–	
Right.	1845.°	4811.°	13.°1
Left.	1660.°	6941.°	23.°4

This shows on the whole a less sensitive right side, or, to put it differently, a more marked negative phototaxis of the left side. How is it when the animals become positively phototactic? Table XVII. gives the average positive response of the right and left sides for series 8 to 18, including series 7*a*, 8*a*, 9*a*, and 10*a*.

TABLE XVII.

SUM OF RESPONSES OF RIGHT AND LEFT SIDES WHEN PHOTOTAXIS IS POSITIVE.

Side turned toward Light.	Total Angular Migration.		Average Positive Phototaxis.
	+	–	
Right.	6850.°	2960.°	7.°68
Left.	5608.°	2190.°	7.°75

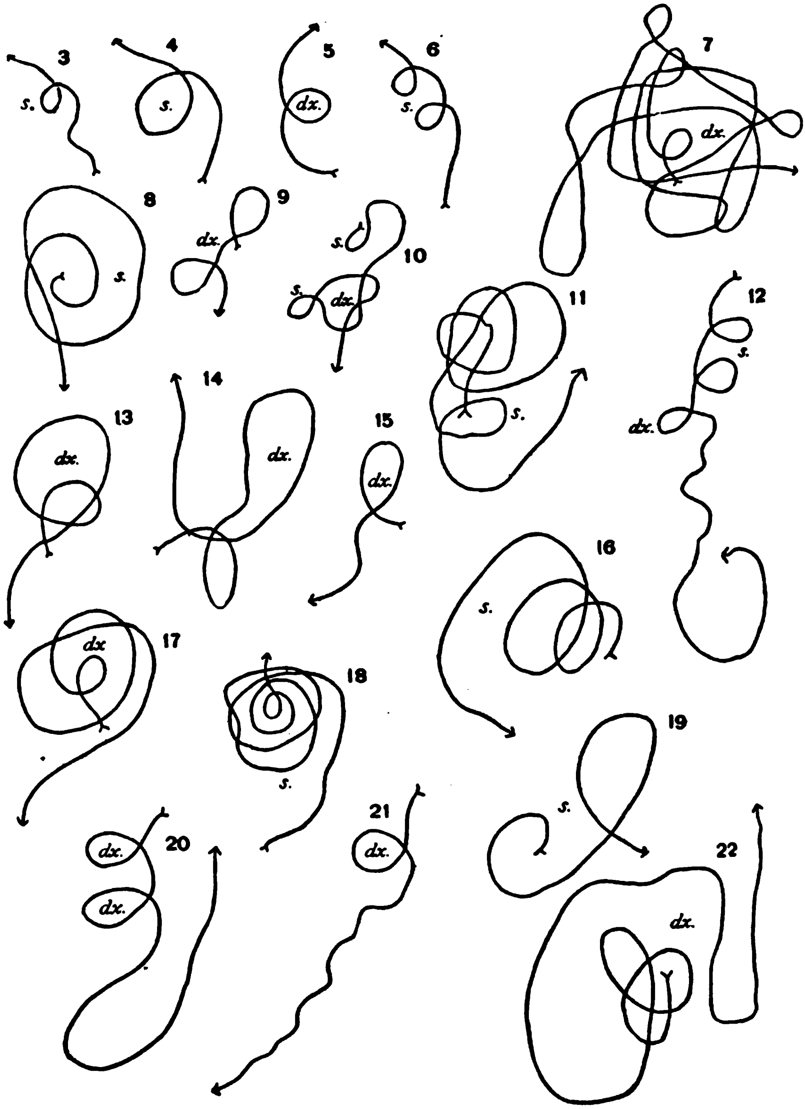
Here an asymmetrical response is less strongly marked. The left side, however, appears on the average to be somewhat more strongly attracted toward the light. The results prove that the asymmetry in response of the right and left sides cannot be wholly due to a tendency to move toward the right, for, if this were so, we should expect an average positive response of the right side as much greater than that of the left side, as the average negative response of the left is greater than that of the right side, for both these would mean a greater movement to the right. These facts curiously suggest that the right and left sides are attuned to slightly different intensities of light. Is this possibly due to ancestral habits of life in which environment, acting unequally on the two sides, produced this difference?

The results obtained for the right and left sides from the experiments in darkness (series 18*a*) are rather puzzling. If the responses are due to some uncontrolled directive stimuli of the kind already suggested, it would seem that the two sides had given opposite responses. As these experiments represent two series taken at different periods, it is the more surprising that they should both show this peculiarity. Again, in the responses to weak candle heat (series 18*b*) the left seems to have been positively, and the right side negatively affected. So far as is known, there was no unequal operation of stimuli on the two sides.

Related to this matter is the question, — Is there any tendency on the part of all slugs to move either to the right or to the left? Individuals were noticed which seemed to have a marked tendency to continue moving toward the right, and there were others which seemed to be as strongly biassed toward the left. Not many seemed entirely indifferent. The total movement of all the slugs in the region of negative response (series 1–8, Table XV.) toward the right side was 8786° (col. 9), and to the left 6471° (col 10). In the positive region (series 8–18, Table XV.), the total migration toward the right side was 8540° (col. 9), and toward the left 8568° (col. 10). Thus, there seems to have been considerably less migration toward the left in the range of negative responses, but only a slightly greater movement toward the left in the region of positive response. In all the 17 series, there was a migration towards the right of 17,326°, and towards the left of 15,039°. That is, there appears on the whole to have been a slightly greater average movement for all slugs toward the right than there has been toward the left. What do we find to be the case with the animals experimented on in the dark? Out of the 120 determinations made on 20 animals in the dark (series 18*a*), the amount of right-hand movement was 2270° and the left-hand movement only 460°. That is, there was nearly five times more migration toward the right than there was toward the left. In series 18*b*, however, there seems to have been a marked preponderance of movement toward the left. From the foregoing experiments, it seems pretty clear that there is a difference in the sensitiveness of the right and left sides. There is also some indication of a slightly greater average tendency to move to the right. But a further study of the undirected movements of slugs in the dark is needed.

Studies have been made by several observers on the undirected movements of a number of different animals, chiefly ants and other insects. In all animals experimented on, there appears to be a tendency to travel in loops or constantly widening spirals. Man, when he loses his way, travels in a circle. Some interesting observations have been made by George and Elizabeth Peckham ('98, pp. 211–219) on the sense of direction in the solitary wasps. When the wasp starts out from its nest, it flies quite around it and gradually circles farther and farther away in a constantly enlarging spiral, sometimes recrossing its path a number of times. The authors' observations show that this action is to enable the wasp to familiarize itself with its surroundings, so that it can find its way home when it so desires. The similar phenomenon observed in other insects, such as ants, is, no doubt, for the same purpose. Davenport ('97, pp. 278–279) in his experiments on Amoebae found that, when their movement was undirected by any external stimulus, they tended to travel in curious spiral loops. Pouchet ('72, pp. 227–228) made observations on the movement of larvae of Musca (Lucilia) caesar in the dark. There is a striking contrast between the paths given by him of the undirected movements and those made in response to the stimulus of light. The tendency to travel in a gradually widening spiral has also been observed by the writer in young frog and toad larvae — before the development of mouth and eyes — when they are dislodged from the support to which they are clinging.

Most of the following experiments on the slug were made in a room about 12 feet square. The floor was sometimes covered with cardboard or paper, but in other experiments was left bare. Heavy curtains were hung in front of the windows and light shut out as completely as possible. The experiments were conducted at night, and the temperature of the room was nearly, if not quite, constant. A slug was put on the floor in the centre of the room and left to itself for two or three hours, sometimes longer. By means of the mucous secretion, which hardened into white, shiny flakes, the exact path of the animal could, in most cases, be easily followed. This path was roughly reproduced by pencil on paper. A number of these paths are given in Figures 3-22, much reduced from the actual space covered. The series here given includes all the animals experimented on, with the exception of three individuals which did not



FIGURES 3-22.

Much reduced copies of the tracks made by slugs (Limax maximus) in the dark. *dx.*, right-handed loops; *s.*, left-handed loops.

give any characteristic paths. Two of the three moved only a short distance in wavy lines without recrossing their paths, and were in poor condition, for they did not go far, and shortly died. One extremely active little individual moved ahead in a straight line quite across the floor, a distance of eight or ten feet. With these few exceptions, it will be seen that there is a very marked tendency to travel in loops. In general, the loops varied in size from a couple of inches in diameter to two feet and sometimes more. The animal generally makes a circle soon after starting out, and then may travel for some distance before again recrossing its tracks. The individuals which did the most looping also showed a tendency, by gradually swinging away from the starting point, to make larger and larger circles. Nos. 7, 8, 11, 13, 14, 16, 17, 19, and 22 all showed this tendency. The smaller individuals usually make the smaller loops, but this is not always the case. Although the paths made by different animals have a very different appearance, they all show the same general looping tendency. It will at once be noticed that all curves are not in the same direction. Some are right-handed loops, others are left-handed, and two cases, Nos. 10 and 12, contain loops of both right and left hand character, or at least indicate a tendency to the formation of such loops. As a rule, however, the individual shows a marked constancy in the character of the loops made. Disregarding the two cases in which there were both right and left hand loops, we have ten individuals with a tendency to circle to the right and eight individuals with just as marked a tendency to circle to the left. This does not indicate a very great preponderance of individuals travelling to the right. If the total space travelled over by all individuals be considered, I think it might show, on the average, a more marked swerving to the right than does a counting of right and left circling individuals, but I have not measured the distances carefully enough to speak confidently on this point. The evidence thus far accumulated in regard to an asymmetrical response of the right and left sides to artificial stimuli points to a greater sensitiveness of the left side, which is perhaps correlated with a *slight* average tendency to move toward the right side more than to the left.

Summary of Part III.

These studies on the light responses of Limax maximus seem to establish the following points: —

(1) The animals are markedly phototactic.

(2) There are individual differences in phototaxis, as there are in geotaxis.

(3) To strong light, slugs, on the average, give a strong negative response.

(4) The degree of response gradually diminishes with the reduction in the strength of the stimulus.

(5) There is a certain strength of light which appears neither to repel nor attract the slug. This may be said to be a neutral stimulus.

(6) Reduction of the intensity of the light beyond the neutral point changes the phototaxis from negative to positive.

(7) The positive response becomes stronger up to a certain degree of intensity.

(8) It then gradually diminishes with decreasing intensity until absolute darkness accompanied by no response is reached.

(9) Slugs are responsive to light stimuli covering a wide range of intensities.

(10) The principal organ of response is probably the eye.

(11) The response is unsymmetrical on the part of the right and left sides of the animal's body. The right side is not as sensitive to stimuli as is the left. On the whole the right side moves through a slightly greater arc in a period of 45 seconds than does the left.

(12) In the dark, other directive stimuli being eliminated, the slug tends to travel in a spiral of gradually increasing radius, though almost invariably producing one or more loops. Some slugs make right-hand loops, others left-hand ones; there is a slightly greater tendency to right-hand circling.

These responses of the slug to touch, gravity, and light-stimuli emphasize the fact that it is an animal's normal environmental conditions which chiefly determine its general response to artificial stimuli. The variations in precision and character of this general response are mainly dependent on certain internal factors, such as the food conditions of the animal, its fear of an enemy, and desire to escape captivity.

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It is a pleasure to acknowledge my indebtedness to Professors E. L. Mark and G. H. Parker for assistance in this investigation.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE.

E. L. MARK, *Director*.

⁂ Abbreviations used :—

B. M. C. Z.	for Bull. Mus. Comp. Zoöl.
P. A. A.	for Proceed. Amer. Acad. Arts and Sci.
P. B. S. N. H.	for Proceed. Bost. Soc. Nat. Hist.

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MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD
COLLEGE. E. L. MARK, DIRECTOR.

No. 128.

A CONTRIBUTION TO THE PHYSIOLOGY OF THE NERVOUS SYSTEM OF THE MEDUSA GONIONEMUS MURBACHII.

PART I.—THE SENSORY REACTIONS OF GONIONEMUS.

By ROBERT M. YERKES.

FROM THE AMERICAN JOURNAL OF PHYSIOLOGY, VOL. VI. NO. VI.

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CAMBRIDGE, MASS., U. S. A.
FEBRUARY, 1902.

Proceedings of the American Academy of Arts and Sciences.

VOL. XXXVII. NO. 21. — APRIL, 1902.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, UNDER THE DIRECTION OF E. L. MARK. — No. 129.

CERTAIN SENSE ORGANS OF THE PROBOSCIS OF THE POLYCHAETOUS ANNELID RHYNCHOBOLUS DIBRANCHIATUS.

BY ADÈLE OPPENHEIMER.

WITH SIX PLATES.

JAN 30 1903

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CERTAIN SENSE ORGANS OF THE PROBOSCIS OF THE POLYCHAETOUS ANNELID RHYNCHOBOLUS DIBRANCHIATUS.

BY ADÈLE OPPENHEIMER.

Presented by E. L. Mark, April 13, 1898. Received February 15, 1902.

THE proboscis of Rhynchobolus dibranchiatus was described by Ehlers ('64–68, p. 670) as "short, thick, club-shaped, with small egg-shaped papillae (compare Plate 1, Fig. 1), and was divided by him (p. 678) into two parts, the "Rüsselröhre," or sheath of the proboscis, and the "Kieferträger," or bearer of the jaws. Before eversion the "Rüsselröhre" is anterior to the "Kieferträger," but when the proboscis is everted (Fig. 1) the latter is anterior. The "Keiferträger" may be subdivided, as Ehlers suggested, into three regions, which in the non-everted state are respectively anterior, middle, and posterior: (1) the anterior has none of the small egg-shaped papillae; (2) the middle region is that supporting the four jaws; and (3) the posterior is, as a rule, not everted, it is the region of the four glands (*gl.*) of the jaws and the remainder of the proboscis following the glands. The boundary between "Kieferträger" and "Rüsselröhre" is marked, according to Ehlers, by the place of attachment to the proboscis of four partial diaphragms, called by him "Lappen" (Fig. 1, *lmn.*).

When cross sections of the everted proboscis are made in the region of the four partial diaphragms (Fig. 2), one encounters in succession in passing from the surface toward the centre (1) a cuticula (*cta.*); (2) an epithelial layer (*e'th.*); (3) a connective-tissue layer (*tis. co'nt.*), in which are embedded eighteen longitudinal nerves (*n. lg.*), and a nerve plexus; (4) a region composed of eighteen longitudinal muscles (*mu. lg.*); (5) a sheet of circular muscles (*mu. crc.*); (6) a fascia or peritoneum (*pi'tn.*)

lining the body-cavity; then in the body-cavity the four partial diaphragms; and finally that part of the proboscis which has not been everted. This consists of nearly the same kinds of layers arranged in the reverse order, namely a peritoneum, circular muscles, longitudinal muscles, nerves, connective tissue, and cuticula.

The epithelial layer directly beneath the cuticula is not mentioned as such by Ehlers. Since it apparently undergoes an interesting metamorphosis, it is worthy of further study.

From the underlying connective-tissue layer eighteen projections of connective tissue pass radially inward between the eighteen longitudinal muscles to the region of the circular muscles. Where the radial projections are continuous with the outer circular portion of the connective tissue the eighteen longitudinal nerves (*n. lg.*) are seen cut crosswise. (Compare Pl. 2, Fig. 10.)

Concerning the structure of these longitudinal nerves I have nothing to add to what Ehlers ('64–68, p. 696) has already pointed out. They are evidently surrounded by a protecting connective tissue, within which lie what are apparently nerve fibres. In preparations fixed in vom Rath's picric-osmic-platinic chloride-acetic mixture, the nervous plasm is flocculent and has shrunken away from the nerve sheath.

From these longitudinal nerves, fibres pass out (Pl. 2, Fig. 10) to form the peripheral nerve plexus, which is embedded in the connective tissue occupying the space between the longitudinal muscles and the cuticula. Other nerve fibres (*n. r.*) starting from the plexus pass radially inward, skirting the longitudinal muscle (Fig. 10); yet apparently they do not innervate the muscles, for I have seen no nerve fibre pass through the sheath enclosing the muscle. Still other radial nerve fibres (*n. r.'*) can be followed from the longitudinal nerves passing through the middle of the radial connective-tissue projections toward the centre of the sections as far as to the membrane immediately superficial to the circular muscles (Pl. 1, Fig. 2; Pl. 2, Fig. 10). In the anterior region, where the four partial diaphragms, the "Lappen" of Ehlers, are attached to the wall of the proboscis, radial nerve fibres occupying the same relative position as those marked in other regions *n. r.'* can be traced into these four pendent structures. Ehlers says concerning these "Lappen" (p. 686): "By means of a fold it [the fascia which invests the surface of all these parts] forms the four 'Lappen,' which are attached at the boundary between 'Rüsselröhre' and 'Kieferträger;' these 'Lappen' therefore possess the fine tense membrane on both surfaces; between lies a fibrous tissue, which is apparently identical with the subcuticular tissue of the sheath of the proboscis, with which, moreover, it is evidently continuous. This tissue . . . consists of a fibrous network, in the meshes of which lie ganglion cells." Further on (p. 696) he says: "The ganglion cells between the leaves of these 'Hautlappen' lie in a single layer and are surrounded by strands of fibres, so that they lie as it were in the meshes of such a net made up of bundles of fibres; however, it seems to me very doubtful whether these strands of fibres which make the meshes are all of nervous nature; on the contrary I believe that the greater mass of this fibrous tissue is identical with that which lies under the chitinous cuticula of the 'Rüsselröhre' and forms the sheath of the longitudinal nerves."

If I understand Ehlers correctly (he has no figures showing these histological conditions), I do not entirely agree with him concerning the structure of the "Lappen." Within the peritoneum I find connective tissue, ganglion cells, and also cells not mentioned by Ehlers (Pl. 2, Figs. 7, 8). These last have an epithelial character; they form, indeed, the main bulk of the lobe, as appears both in material prepared in the vom Rath mixture and in two haematoxylin preparations made from material fixed respectively in corrosive sublimate and in sublimate-acetic. The "Fasergewebe" of Ehlers I consider nervous in large part. Almost all of the fibres (Fig. 8) surround, not the ganglion cell, as one might infer from his description, but its nucleus, and pass out at one pole of the cell body to the longitudinal nerves of the proboscis.

Finally, nerve fibres from the longitudinal nerves and from the peripheral nerve plexus can be traced out peripherally into the small papillae which are thickly distributed over the surface of the "Russelröhre."

Through the kindness of Mrs. Margaret Lewis Nickerson, who suggested to me the subject of the present paper, I was able to begin my study of the distribution of the sensory papillae of the proboscis on a preparation of the cuticula already made by her. The cuticula had been prepared by a method which was first employed by Mrs. Nickerson. All my subsequent preparations of the cuticula of other individuals were secured by the same method, which was as follows: The worm, after being narcotized in a mixture of sea-water and alcohol, was placed in a ten per cent solution of common salt until it was evident that its skin was loosened from the body. A cut was then made through the cuticula along a longitudinal line of the body, and the animal placed in tap-water. After the salt had been thoroughly washed out, the worm was cut transversely into pieces short enough for the cuticula to be mounted conveniently on a slide. The cuticula was next peeled off with needles and floated upon glass slides. These preparations were ready for study as soon as they were dry.

The whole surface of the proboscis, except the part which is most anterior in the usual state of eversion (Fig. 1), is covered with conical or thimble-shaped papillae, which are arranged on the summit of transverse folds (Pl. 5, Figs. 32, 33). In general the axes of the papillae are perpendicular to the surface of the proboscis, or are directed outward and either slightly backward or slightly forward. The rows of papillae are as a rule separated from each other by regular intervals, but sometimes there is an anastomosis (Fig. 32) of the folds from which these organs project. The folds follow one another closely, and there are one or two rows of papillae to each fold. At the posterior part of the everted proboscis the transverse rows are divided into eighteen longitudinal groups (Fig. 33); the interspaces correspond to the position of the eighteen longitudinal nerves. Otherwise the arrangement and frequency of these organs is the same from the anterior to the posterior end of the proboscis, and there is no other evidence of special grouping in any part.

The papillae are more or less ovoid or conical. On a proboscis about 5½ mm. in diameter at the anterior end, they were found to be about 80 μ in height and about 35 μ in diameter at the thickest part.

The cuticula of the proboscis passes over each papilla, but is here reduced to about two-thirds the thickness it has elsewhere. The cuticula of the posterior face of each papilla is coarsely corrugated. The ridges are most clearly seen in preparations of removed cuticula (Pl. 6, Fig. 34), or in sections stained in Kleinenberg's haematoxylin (Pl. 1, Fig. 6; Pl. 3, Fig. 13). Though varying in number in different papillae, the ridges show considerable regularity of form and arrangement, for the outlines produced by them are always rather sharply bent in a region corresponding with the middle of the posterior face of the papillae, so that the surface view of that face shows a series of V-shaped outlines, like the longitudinal section of a nest of funnels, the apices of the V's being directed toward the base of the papilla. Sometimes, however, there is an anastomosis of the folds (Pl. 3, Fig. 13). Ehlers (p. 679) says of this species of Rhynchobolus that the cuticula of the papillae has "fine folds, which, like those of the gills, occur in spiral lines, surrounding the papilla, or more rarely, standing out as sharply projecting ridges." Concerning the gills he says (p. 676): "The chitinous covering possesses at fairly regular intervals furrows which pass around the circumference spirally; their significance probably consists in their laying the gill into definite folds when it collapses and withdraws into the parapodial pouch." Whatever may be the condition in the case of the gills, the furrows of the papillae do not encircle those organs, for I have found that they exist on the posterior face of the papilla only. That the function of the furrows of the papillae is similar to that suggested by Ehlers for those of the gills, namely to determine the place of folding when the organs are retracted, may well be questioned, for there is no evidence that the papillae are ever retracted; there are no muscles to effect contraction, nor have I ever found the organs in a retracted condition.

The papillae have been studied in sections fixed in a mixture of corrosive sublimate and acetic acid and subsequently stained in Kleinenberg's haematoxylin; in sections fixed in corrosive sublimate and stained in iron haematoxylin; in preparations fixed in vom Rath's ('95, p. 282) picric-osmic-platinic chloride-acetic mixture (to which tap-water was sometimes added); and in methylen-blue preparations. The sections stained in iron haematoxylin I prepared, through the kindness of Professor Lloyd, in the laboratory of the Teachers College, Columbia University.

The living substance of the papillae appears to consist of either four or five cells, which are, to judge from the nuclei, of two kinds. Two of the nuclei (Pl. **1**, Fig. 3; Pl. **3**, Fig. 16, *nl. ba.*) found in the papillae are basal in position and larger than the others; the remaining two or three (*nl. ax.*) are nearer the apex of the papilla and also usually more nearly axial in position (Pl. **1**, Figs. 3, 4; Pl. **2**, Figs. 9*a*, 9*b*, 11; Pl. **3**, Figs. 16, 17; Pl. **4**, Figs. 26, 28, 30). The boundaries of the two cells to which the two basal nuclei belong cannot be made out by any process that I have employed.

In preparations made with vom Rath's mixture, the protoplasmic contents of the papilla are distinctly vacuolated. The vacuoles are also seen with nearly equal distinctness in the methylen-blue preparations, but not quite so clearly in sections stained with iron haematoxylin or with Kleinenberg's haematoxylin. The vacuoles are merely clearer, usually roundish, regions, which stand out distinctly, in contrast to the deeply stained granular or fibrous surrounding substance, and are quite variable in size, as is to be seen in Pl. **3**, Figs. 18, 20; Pl. **4**, Figs. 22, 25, 29. I believe that some of the more elongated vacuoles and the clusters of the more rounded ones in the region of the central nuclei (Figs. 22, 29), and perhaps a lighter coloring of the axial region of the papilla (Pl. **1**, Fig. 4; Pl. **2**, Fig. 11), gave rise to the following opinion expressed by Ehlers (p. 679): "There lies under the chitinous covering a thin sheet of finely granular substance, *which in the papilla appears to surround a narrow cavity*, and there is connected with this sheet a thick layer of fibrous tissue."

Connective-tissue fibres pass from the connective tissue of the proboscis into the papillae (Pl. **1**, Fig. 4; Pl. **2**, Fig. 11; Pl. **3**, Figs. 12, 19, 20; Pl. **4**, Fig. 27); as a rule, these could not be traced more than half-way to the apex of the papilla, but sometimes the contents of the papilla, in great part or entirely, looked fibrous (Pl. **1**, Fig. 4; Pl. **3**, Figs. 12, 15, 19). These fibres of the papilla are, as Ehlers says, in close connection with a finely granular substance. There is a particularly dense and deeply stained layer of this finely granular substance immediately under the cuticula (Pl. **1**, Figs. 3, 4; Pl. **2**, Fig. 11; Pl. **3**, Fig. 16; Pl. **4**, Fig. 30); it surrounds not a cavity, but a central region in which there is a little granular substance and in which there are many vacuoles. At one point of the base of the papilla, where the connective tissue enters (Pl. **1**, Fig. 4; Pl. **2**, Fig. 11), and again at one point near the apex, apparently in the region of the sensory termination of the papilla (Pl. **4**, Fig. 30*c*), there is a break in the dense layer of finely granular substance.

Of the two basal nuclei (*nl. ba.*) one is near the anterior, the other near the posterior face of the papilla (Pl. **2**, Fig. 9*b*). They are spheroidal or ellipsoidal, and contain small irregularly scattered chromatin granules in large numbers; but in preparations stained in haematoxylin (Pl. **1**, Fig. 3; Pl. **2**, Figs. 9*b*, 11; Pl. **3**, Figs. 16, 17) they appear less deeply colored than the remaining nuclei.

The more distal nuclei (*nl. ax.*) are more elongated, being ellipsoidal or spindle-shaped. They present an elliptical outline whether seen in sections perpendicular to the axis of the proboscis (Pl. **1**, Figs. 3, 4; Pl. **2**, Fig. 11; Pl. **4**, Fig. 30*c*), in longitudinal sections of the proboscis passing through the axis of the papilla (Fig. 28), or in sections perpendicular to the axis of the papilla (Pl. **2**, Fig. 9*a*; Pl. **3**, Figs. 16, 17). The outline may be more or less pointed at one end, and is more nearly circular in the sections perpendicular to the axis of the papilla than in those parallel to the axis. The deeply staining granulations of the distal, or axial, nuclei are larger and not less numerous than those of the basal nuclei; and it is perhaps for this reason that the first-named nuclei appear more deeply stained than the basal ones. The granulations of the axial nuclei are also more evenly distributed. Both kinds of nuclei have a clearly defined nuclear membrane. In the preparations fixed in sublimate-acetic and stained in Kleinenberg's haematoxylin, I have seen a nucleolus in the basal nucleus only, and here only occasionally (Pl. 3, Fig. 16; Pl. 4, Fig. 24). Sometimes, though rarely, there are in a basal nucleus *two* larger granulations (Pl. 2, Fig. 11; Pl. 4, Fig. 30*c*), which may perhaps be entitled to rank as nucleoli. In preparations stained in iron haematoxylin and in those fixed in vom Rath's mixture the nucleolus is regularly seen with great distinctness near the centre of the basal nucleus (Pl. 2, Fig. 9*b*; Pl. 4, Fig. 26–28). The nucleolus is not infrequently surrounded by a light area.

From the different effects produced on the two kinds of nuclei by haematoxylin and by methylen blue, it is fair to conclude that the cells to which the basal nuclei belong are very different from those of the apical nuclei, and that they have nothing to do directly with the nervous system. They are evidently indifferent subcuticular cells, which probably have the same functions as the cover cells of more complicated sensory organs.

The central elongated nuclei found in haematoxylin preparations, judging from their position, evidently correspond to the two or three spindle-shaped cell bodies which appear in methylen-blue preparations.

"I have not succeeded," says Ehlers (p. 690), "in finding proof positive that there are nerves in the fibrous tissue which enters the papilla from the common subcuticular layer." What Ehlers was unable to find, I have, by the use of improved histological methods, succeeded in demonstrating with entirely satisfactory clearness. The spindle-shaped cells are evidently nerve cells of sensory function. For, on the one hand, the basal end is connected with one of the eighteen longitudinal nerves of the proboscis by a nerve fibre passing to that nerve, either directly or, through the intervention of the peripheral nerve plexus, indirectly; and on the other hand the peripheral end tapers toward the apex of the papilla, where it terminates in a sensory structure, the precise nature of which it is difficult to make out.

Each of the sensory cells of the papilla has the form of an elongated spindle tapering at its free end to a delicate fibre-like structure, and continuous at its basal end with a fibre traceable to a nerve trunk. This spindle-shaped enlargement, or cell body, lies in the axis of the papilla and about midway between its base and apex. An exception to this rule regarding the position of the cell body is seen in Figure 20 (Pl. 3), where the cell seems to have a basal position. I am, however, in doubt as to whether the sensory cells in this case are actually basal in position, or whether the appearance may not be due to an accidental staining of parts adjacent to the nerve fibres, — a sort of extravasation, — accompanied by a failure to stain on the part of the real cell body and the more distal portions of the sensory cell. The spindle-shaped enlargement is sometimes stained uniformly, but more often the staining is irregular and blotchy; in some cases a nucleus is to be distinguished near the middle of the cell body in the widest part of the spindle, which it almost completely fills. In one case (Pl. **3**, Fig. 14) the nucleus was sharply differentiated from the cell body, which was not at all blotchy, but distinctly fibrous and sparsely granular.

From the distal end of the spindle-shaped cell body there passes off a fibre that, I believe, breaks up into a number of fibrils, each of which seems to me to end in a disc (Pl. **1**, Fig. 5; Pl. **3**, Fig. 14). In Figure 31 (Pl. **4**), the fibrils are quite clearly recognizable; in Figures 25 and 29 (Pl. **4**), though distinguishable, they are not so evident. The terminal discs (Pl. **3**, Fig. 18; Pl. **4**, Figs. 25, 29) may, it is true, be artefacts; but the frequency of their occurrence and the similarity of their appearance seem to me to be arguments against that supposition. Sometimes the blue is deposited in great amount around this bunch of fibrils (Pl. **3**, Figs. 12, 15, 18; Pl. **4**, Fig. 29), but in other cases it has failed entirely to stain the portion of the sensory cell that is distal to the spindle-shaped enlargement. On the other hand, there are cases in which the peripheral part of the distal portion of the sense cell has been differentiated by staining in haematoxylin (Pl. **2**, Fig. 11, not well brought out in the figure). In the case in which I have seen fibrils with their terminal discs most distinctly (Pl. **3**, Fig. 14), the discs at the ends of the fibrils are at the surface of the papilla outside the cuticula; in other preparations, the fibrils seem not to pass through the cuticula, but to end at its deep surface. It is probable that in most cases the cuticula has been artificially separated from the protoplasmic mass of the papilla, and that normally the fibrils pass to the surface of the papilla.

The connection of the cell body with one of the eighteen longitudinal nerves of the proboscis is often to be traced on a single thick section. The process which the cell body sends centripetally either joins a longitudinal nerve directly, or enters the peripheral nerve plexus, which in turn joins the longitudinal nerve (Pl. **2**, Fig. 10; Pl. **3**, Fig. 19). The basal end of each of the two or three cell bodies of the papilla seen in methylen-blue preparations (Pl. **3**, Figs. 12, 14, 20; Pl. **4**, Figs. 23, 25, 29) is prolonged into a slender nerve fibre. While the fibre belonging to one of the cells of a papilla bends to the left when it joins the nerve plexus, that belonging to another cell of the same papilla may bend to the right, as is to be seen in Figures 10 (Pl. 2), 12, and 19 (Pl. 3). Occasionally the fibres twist around each other, and there is sometimes to be found an appearance which suggests anastomosis of these fibres, but focusing shows that in a great number of such cases the fibres cross without touching each other; in still other cases (Pl. 3, Figs. 12, 19) the blue staining is not confined to the fibres, and this makes the following out of the fibres more difficult.

The condition shown in Figure 15 (Pl. 3), which seems to be an exception to the rule that the basal end of each spindle-shaped cell body tapers into a nerve fibre, is probably the result of the well-known capriciousness of methylen-blue staining. In no case have I seen a nerve fibre arise from an abruptly rounded basal end of one of these sensory cells, but the cell body seems always to taper gradually into the nerve fibre. There are, however, quite a number of cases in which the inner end of the cell body does not simply taper into a single nerve fibre, but in which it is prolonged into a few processes which ultimately unite to form the fibre (Pl. 4, Figs. 23, 25).

These nerve fibres on their way to the longitudinal nerves often show at intervals those characteristic swellings, or varicosities, which have been so frequently figured in recent works on nerve fibres treated either by the methylen-blue or the Golgi methods.

SUMMARY.

The papillae of the proboscis of Rhynchobolus are sensory organs. They are considered to be sensory on the following grounds: —

1. The papillae are well differentiated organs.
2. They are found over almost the entire surface of the everted proboscis.
3. They are elevated above the surrounding surface.
4. The cuticula which passes over each papilla is reduced to about two-thirds the thickness it has elsewhere on the proboscis.

It should be mentioned that the cuticula of the posterior face of each papilla is coarsely corrugated, but the significance of this wrinkling is unknown.

5. There are two or three spindle-shaped cells in a papilla, each of which terminates — either below the cuticula or more probably at the very apex of the papilla — in what is clearly a sensory structure, and each of these cells tapers gradually at its base into a nerve fibre. These

nerve fibres are connected either directly or indirectly — through the intervention of a peripheral nerve plexus — with the eighteen longitudinal nerves of the proboscis.

6. There are two basal nuclei that belong to cells which probably have the function of cover cells.

It remains to be said that there enter each papilla besides nerve fibres, connective-tissue fibres. These latter are found in close connection with a finely granular substance, of which there is a particularly dense and deeply staining layer immediately under the cuticula. Standing out in contrast to the deeply stained granular or fibrous surrounding substance are the clear, generally rounded vacuoles.

If there is any differentiation in function between papillae, it is not correlated with any pronounced difference in structure.

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EXPLANATION OF PLATES.

Abbreviations.

coel.	Coelom, body-cavity.
cta.	Cuticula.
cta. + *e'th.*	Cuticula and epithelium.
gl.	Gland.
gna.	Jaw.
lmn.	Lemniscus.
mu. crc.	Circular muscle.
mu. lg.	Longitudinal muscle.
n. crc.	Circular nerve.
nl. ax.	Axial nucleus.
nl. ba.	Basal nucleus.
n. lg.	Longitudinal nerve.
n. r.	Radial nerve fibre skirting longitudinal muscle.
n. r.'	Radial nerve fibre passing directly to the membrane superficial to the circular muscles.
pap.	Papilla.
pi'tn.	Peritoneum.
tis. co'nt.	Connective tissue.

In many figures not only the papilla is shown, but also a portion of the underlying parts.

PLATE 1.

FIG. 1. Longitudinal section of the everted proboscis showing: (1) the sheath of the proboscis; (2) the bearer of the jaws and its subdivision; and (3) the lemniscus (*lmn.*), which marks the boundary between (1) and (2).

Narcotized in a mixture of sea-water and alcohol; fixed in Müller's fluid; stained with Beale's ammonia carmine. × *circa* 11.

FIG. 2. Cross section of the partially everted proboscis in the region of the four lemnisci (*lmn.*), showing, among other things, a diagrammatic representation of the papillae and the connection of their sensory cells with the circular and the longitudinal nerves, and also the nerve fibre (*n. r.'*) passing to the membrane which invests the circular muscles.

Chloroform, methylen blue, Bethe's ammonium molybdate for invertebrates. × 14.5.

FIG. 3. Papilla from a cross section of the proboscis, showing two "basal" and three "axial" cell nuclei.

Sea-water and alcohol, sublimate-acetic, Kleinenberg's haematoxylin. × 675.

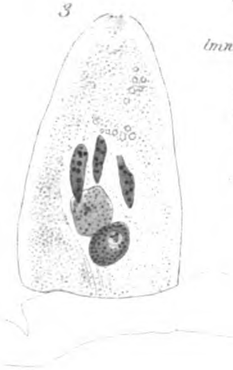
FIG. 4. Longitudinal section of a papilla, from a cross section of the proboscis, showing the two axial nuclei and one of the two basal nuclei, also fibrous structures entering the base of the papilla. Treatment the same as in Fig. 3. × 585.

FIG. 5. Longitudinal section of a papilla, from a sagittal section of the proboscis, showing two sensory axial cells with peripheral sensory termination and prolongation of the basal end of each into a slender nerve fibre.

Chloroform, methylen blue, Bethe's ammonium molybdate for invertebrates. × 650.

FIG. 6. Papilla from cross section of proboscis viewed from behind, showing the corrugations of the cuticula on the posterior face of the papilla, and in optical section the two zones of living substance together with one of the basal nuclei.

Sea-water and alcohol, sublimate-acetic, Kleinenberg's haematoxylin. × 585.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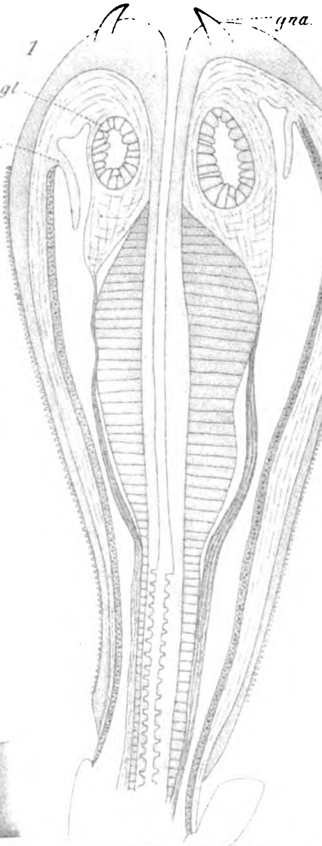
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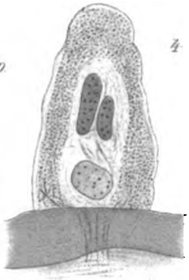
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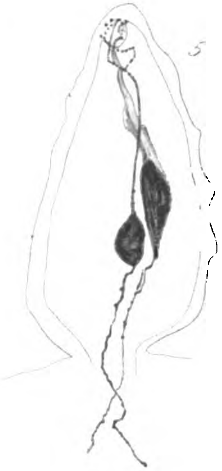
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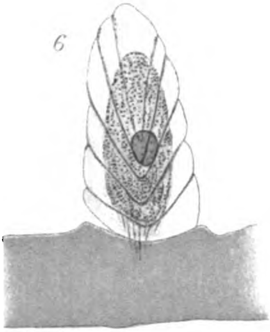
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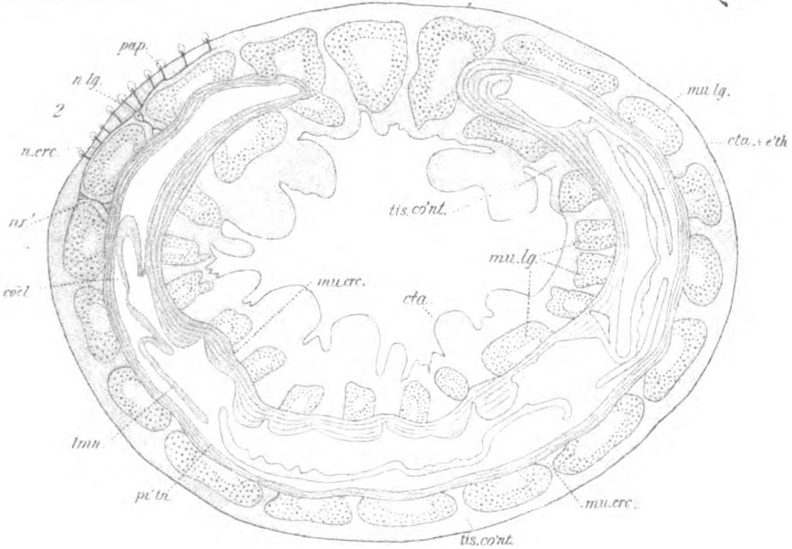
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PLATE 2.

FIG. 7. Portion of cross section of proboscis, showing structure of lemniscus. Sea-water and alcohol, vom Rath's mixture. × 200.

FIG. 8. Part of Fig. 7 enlarged. × *ca.* 400.

FIGS. 9*a*, 9*b*. Sections of a papilla perpendicular to its axis. Figure 9*a* represents the more distal of the two sections, and shows the form and position of the three axial nuclei; Figure 9*b* shows the two basal nuclei. The anterior face of the papilla is directed toward the top of the plate in both cases.

Sea-water and alcohol, corrosive sublimate, iron haematoxylin.

FIG. 10. Portion of the cross section of an everted proboscis, showing one of the eighteen longitudinal nerves (*n. lg.*) cut crosswise, the peripheral nerve plexus, the union of the centripetal processes from the sense cells with the longitudinal nerve (in the case of the third papilla from the upper margin of the Figure, one of the two nerve fibres bends to the left when it enters the nerve plexus, the other to the right), a radial nerve (*n. r.*) following the surface of the longitudinal muscle (this is sketched in from an adjacent section), and another radial nerve (*n. r.'*) passing directly to the membrane which is immediately superficial to the circular muscles.

Chloroform, methylen blue, Bethe's ammonium molybdate for invertebrates. × 145.

FIG. 11. Papilla from a cross section of proboscis showing one of the basal and one of the axial nuclei; there are two large granulations in the basal nucleus.

The differentiation of the distal portion of the sense-cell is not well shown.

Sea-water and alcohol, sublimate-acetic, Kleinenberg's haematoxylin. × 460.

OPPENHEIMER–SENSE ORGANS RHYNCHOBOLUS. PLATE 2

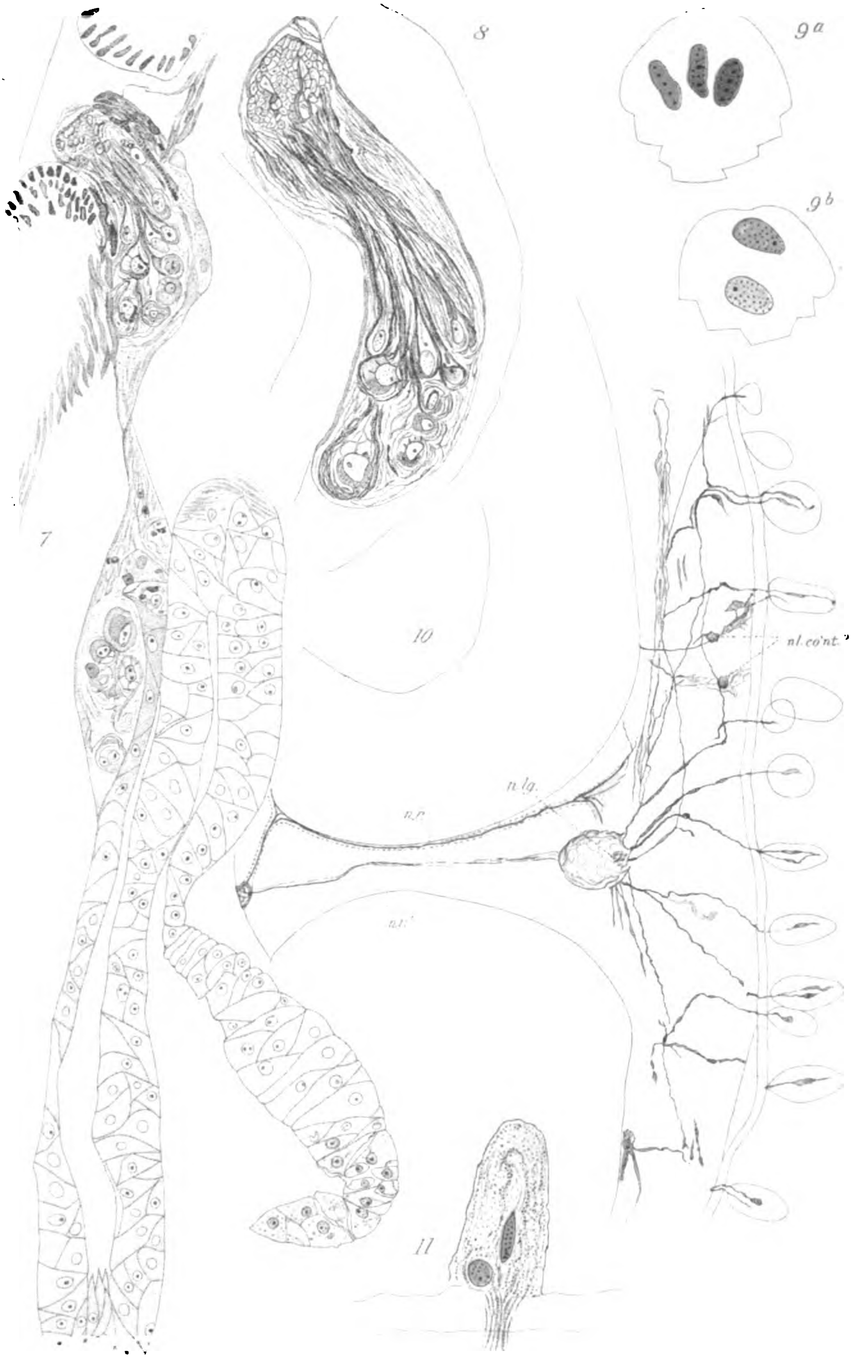

PLATE 3.

FIGS. 12, 14, 15, 18, 19, 20. Preparations made by use of chloroform, methylen blue, and Bethe's ammonium molybdate for invertebrates.

FIGS. 13, 16, 17. Prepared by use of sea-water and alcohol, sublimate-acetic, Kleinenberg's haematoxylin.

FIG. 12. Papilla from cross section of proboscis, showing connective-tissue fibres passing into the papilla; deep coloration of terminal fibrils; the nerve fibres bending in opposite directions where they enter the nerve plexus. × 680.

FIG. 13. Papilla from cross section of proboscis, showing corrugations of posterior face of papilla, and the outline of one of the basal nuclei. × 460.

FIG. 14. Papilla from sagittal section of proboscis; the two sensory (axial) cells, their peripheral terminations, and their proximal nerve-fibre prolongations stained blue.

The nucleus of one of the sensory cells more deeply stained than the cell body. Cuticula distended and detached from substance of the papilla by treatment. × 710.

FIG. 15. Papilla from cross section of proboscis, showing deeply stained axial body, from which a single peripheral, deeply stained process extends to the apex of papilla, where it terminates in a specialized and stained area; the contents of the papilla in great part fibrous. × 1020.

FIG. 16. Somewhat oblique cross sections of two papillae from a cross section of the proboscis. In one papilla are two basal nuclei and a part of one of the axial nuclei; in the other the three axial nuclei cut crosswise. × 670.

FIG. 17. Cross sections of two papillae from a cross section of proboscis. In one are seen two axial nuclei, each surrounded with a clear area; in the other a basal nucleus and portions of two axial (?) nuclei. × 670.

FIG. 18. Papilla from cross section of proboscis. The two sensory cells are stained throughout; their distal prolongations have a more or less spiral course and terminate in a cluster of discs at the apex of the papilla. Vacuoles large. × 715.

FIG. 19. Papilla from cross section of proboscis, showing that where the centripetal fibres from two sensory cells meet the nerve plexus, one bends to the right, the other to the left. × 725.

FIG. 20. Papilla from cross section of proboscis, showing the basal position of the sensory cell body (?); the basal end of each sensory cell is prolonged into a slender nerve fibre. × 682.5.

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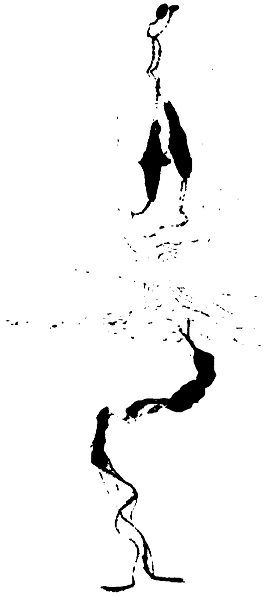

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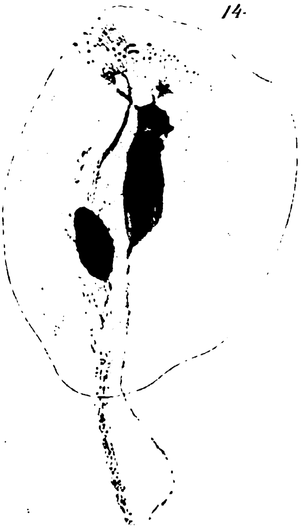

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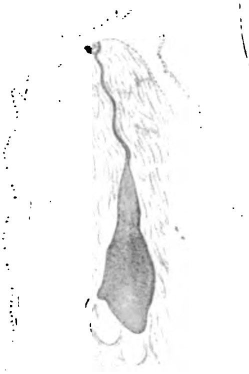

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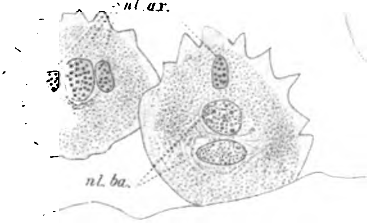

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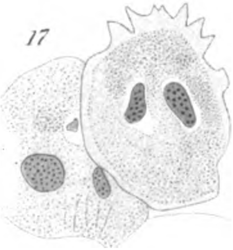

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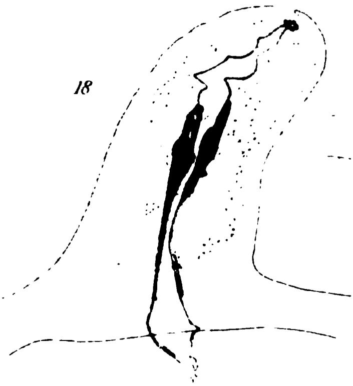

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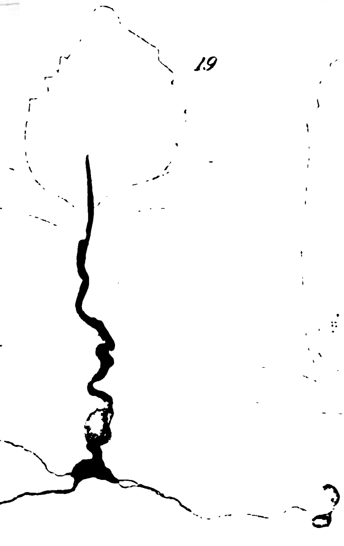

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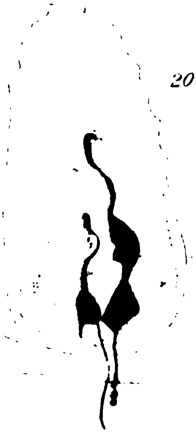

A.O. del.

B. Meisel lith. Boston.

PLATE 4.

Figs. 21–23, 25, 27, 29, 30. Longitudinal sections of papillae from cross sections of proboscis.

Figs. 21–23, 25, 29. Preparations made by use of chloroform, methylen blue, Bethe's ammonium molybdate for invertebrates.

Figs. 24, 30. Preparations made by use of sea-water and alcohol, sublimate-acetic, Kleinenberg's haematoxylin.

Fig. 21. Three sensory cells, two showing peripheral fibres and terminations. × 715.

Fig. 22. Papilla showing a row of axial vacuoles.
Sea-water and alcohol, Müller's fluid, Beale's ammonia carmine.

Fig. 23. The nuclei of the two sensory cells distinguishable from the cell body by their deeper stain. Peripheral and proximal fibres stained. × 710.

Fig. 24. Basal nucleus of a papilla showing a large single nucleolus. × 670.

Fig. 25. Highly vacuolated papilla, fibrils and discs of the sensory termination stained blue, the deep ends of each of the sensory cells prolonged into a few processes, which unite to form the single nerve fibre. × 700.

Fig. 26. Cross section of a small papilla, showing a nucleolus in each basal nucleus.
Sea-water and alcohol, vom Rath's mixture. × 680.

Fig. 27. Papilla showing one of the basal nuclei with large nucleolus, and the passage of connective-tissue fibrils into the papilla.
Sea-water and alcohol, vom Rath's mixture.

Fig. 28. Papilla from sagittal section of proboscis, showing three axial nuclei and two basal nuclei.
Sea-water and alcohol, sublimate, iron haematoxylin.

Fig. 29. Papilla from cross section of proboscis, showing numerous small vacuoles, fibrils and discs of sensory termination. The basal end of each sensory cell is prolonged into a slender nerve fibre. × 730.

Figs. 30*a*–30*d*. Four successive sections from a single papilla.

Fig. 30*b* shows one of the basal nuclei; Fig. 30*c*, the other basal nucleus and the two axial nuclei.

In the region of the apex of the papilla, there is an interruption in the cortical layer of finely granular substance, not well shown, and the region is traversed by fine fibres. × 585.

Fig. 31. Fibrils from the peripheral termination of a sensory cell.

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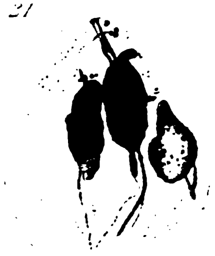

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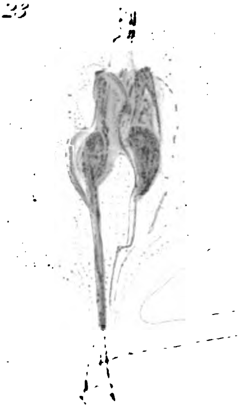

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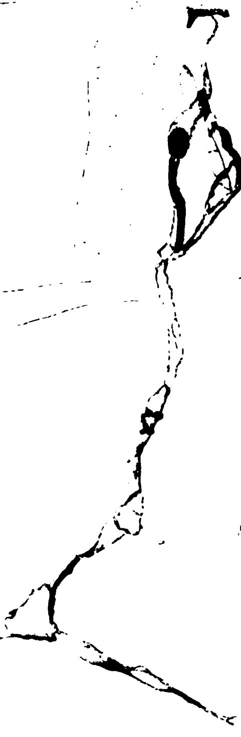

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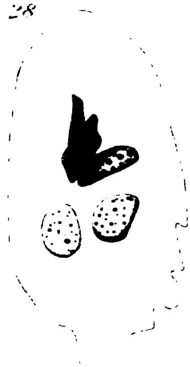
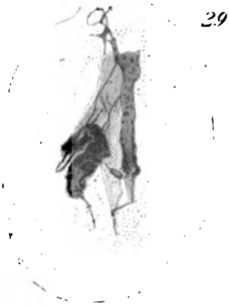
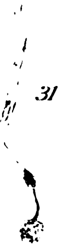
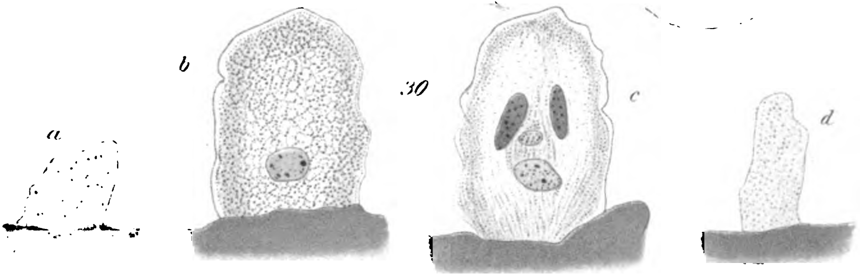


29


31


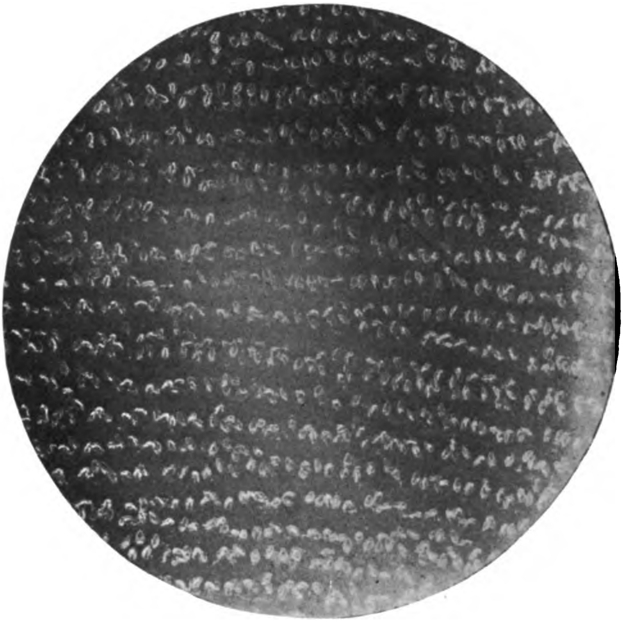
30


A.O. del. A. Hoen & Co. Balt.

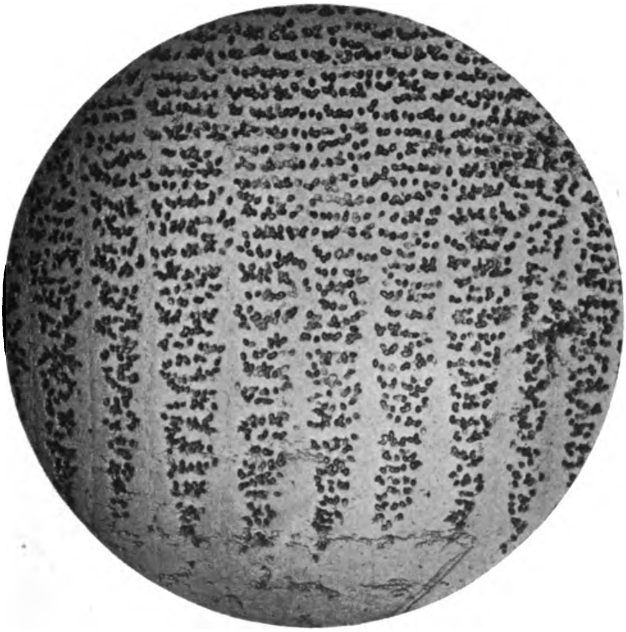
PLATE 5.

FIG. 32. From a photograph of the cuticula of the proboscis stripped by maceration (consult text, p. 555) and mounted on glass slide. The part of the figure nearest the top of the plate is toward the anterior end of the everted proboscis. To show the arrangement of the papillae in transverse rows. × 22.5.

FIG. 33. From a photograph of a preparation similar to that of Fig. 32, showing the appearance of the cuticula and attached papillae near the posterior end of the everted proboscis. Nine of the eighteen longitudinal columns of papillae are shown. × 18.5.



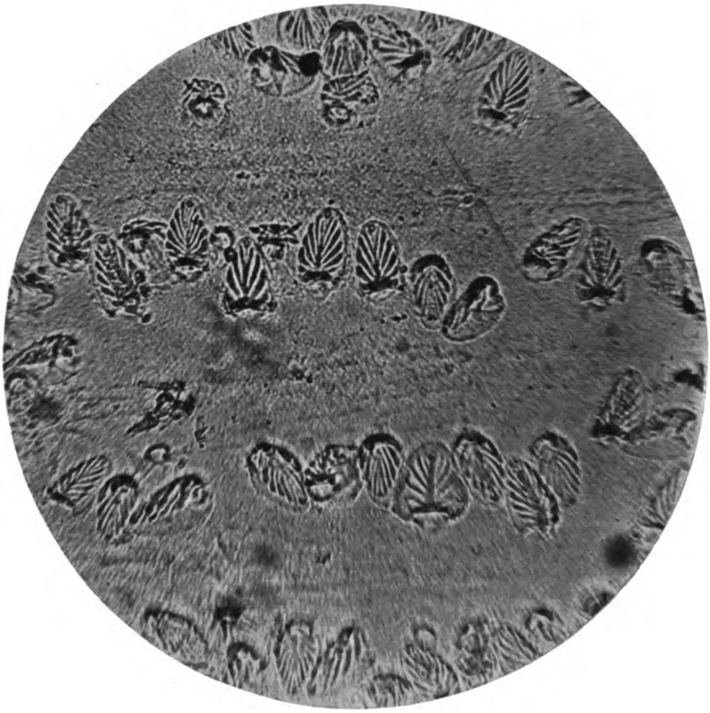
32



33

PLATE 6.

FIG. 34. Highly magnified view of portions of four transverse rows of papillae, to show the corrugations of the flattened and dried papillae, and the circular wall and pit of the cuticula at the apex of the papilla, marking the position of the termination of the sensory cells. × 110.



34

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E. L. Mark, *Director*.

⁂ Abbreviations used : —

B. M. C. Z. . . . . for Bull. Mus. Comp. Zoöl.
P. A. A. . . . . for Proceed. Amer. Acad. Arts and Sci.
P. B. S. N. H. . . . . for Proceed. Bost. Soc. Nat. Hist.

1. **Barnes, W.** — On the Development of the Posterior Fissure of the Spinal Cord, and the Reduction of the Central Canal, in the Pig. P. A. A. **19** : 97–110. 3 pls. 1884.
2. **Tuttle, A. H.** — The Relation of the External Meatus, Tympanum, and Eustachian Tube to the First Visceral Cleft. P. A. A. **19** : 111–132. 2 pls. 1884.
3. **Ayers, H.** — On the Development of Oecanthus niveus and its Parasite, Teleas. Mem. Bost. Soc. Nat. Hist. **3** : 225–281. 8 pls. Jan., 1884.
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6. **Reighard, J.** — On the Anatomy and Histology of Aulophorus vagus. P. A. A. **20** : 88–106. 3 pls. Oct., 1884.
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9. **Fewkes, J. W.** — Report on the Medusae collected by the U. S. Fish Commission Steamer Albatross in the Region of the Gulf Stream in 1883–'84. Ann. Rep. Comnr. Fish and Fisheries for 1884, 927–980, 10 pls., 1886.
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59.13.4: 18.8: 7.56

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR.

No. 130.

CHANGES ACCOMPANYING THE MIGRATION OF THE EYE AND OBSERVATIONS ON THE TRACTUS OPTICUS AND TECTUM OPTICUM IN PSEUDOPLEURONECTES AMERICANUS.

BY STEPHEN R. WILLIAMS.

WITH FIVE PLATES AND SEVEN TEXT FIGURES.

FROM THE BULLETIN OF THE MUSEUM OF COMPARATIVE ZOÖLOGY
AT HARVARD COLLEGE, VOL. XL., No. 1.

CAMBRIDGE, MASS., U. S. A.
MAY, 1902.

JAN [illegible] 1903

No. 1 — CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, UNDER THE DIRECTION OF E. L. MARK, No. 180.

Changes accompanying the Migration of the Eye and Observations on the Tractus opticus and Tectum opticum in Pseudopleuronectes americanus. By Stephen R. Williams.

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I. Introduction.

The strange want of symmetry in the head region of flounders has attracted much attention especially because in adults both eyes occupy the same side of the head. The peculiarity is the more remarkable because, for some time after hatching, the eyes and all other parts of the head are as symmetrical as in any other fish, and consequently this asymmetrical condition is brought about afresh in the individuals of each generation, instead of once for all, as is the case with most variations.

Regarding the migration of the eye, with a single exception (Pfeffer, '86, '94), only such phenomena have been recorded as can be observed from surface study or dissections. It has seemed desirable therefore to learn from careful preparations of specimens in transition stages whether there was merely a mechanical twisting of the facial region in an otherwise normal fish, or a more elaborate rearrangement of the parts with reference to each other, and especially whether any histological changes accompany the more obvious external modifications.

II. Material.

The most of my work has been on the so-called winter flounder (Pseudopleuronectes americanus Walbaum), a dextral flatfish, but I have also used for the sake of comparison a sinistral species, the sand-dab (Bothus maculatus Mitchill).

My material was all collected at Wood's Hole, Mass., during the years 1898 and 1899. I obtained a series of developing eggs and young Pseudopleuronectes from the hatchery of the United States Fish Commission in April, 1898. Adult fishes can be taken by nets at any time through the year. The larval stages at or about the time of the migration of the eye are to be obtained during the month of June only. Early in the month only a few are at the point of assuming the adult position, and after June 20th, all the fish of this species taken were already metamorphosed.

These larvæ were caught by surface towing with a coarse scrim tow-net near the wall of the "outer basin" of the U. S. F. C. wharf during the rising tide. They are most abundant on clear days when the wind is on shore and the tide comes in from the east. On very calm or very rough days they are not plentiful. My most successful skimmings were made early in June, and twice I obtained as many as 100 young fish during the inward flow of the current (3–4 hours). I was able to save a few of the young fish alive by frequently emptying the tow-net and placing the uninjured specimens in as pure water as possible.

In the summer of 1898 the sand-dab larvæ were taken more abundantly than the winter flounders, while in 1899 the winter flounders were about ten times as numerous as the sand-dabs.

I kept the young fish in the "outer basin"[1] in large lamp chimneys, which were made into separate aquaria by tying netting over the ends and were supported by a floating frame. After they had remained here for a time they were removed to the laboratory and kept under observation in running water.

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[1] The granite inclosure for the protection of smaller boats belonging to the United States Fish Commission is divided by projecting parts of the dock into the "inner" and "outer" basin. There are numerous openings in the stone walls to allow the free circulation of the water, and near one of these the float was moored, thus securing as nearly normal conditions of water and food as consistent with protection from violent wave action.

The period at which the eye turns is one of great mortality among the young fish captured, so that most of those in this stage died before removal from the net. Since there is as yet no bony orbit, the eyes are absolutely unprotected. As the eye which is to change its relative position must for a time be on the dorsal side of the head, held in position merely by the skin and a limited amount of connective tissue, it is not strange that in a number of instances young fish were taken alive which had lost the migrating eye some time before their capture.

The actual turning is a comparatively rapid process in the species I have observed, though, as will be seen later, a long preparation is made for it. For instance, those fishes taken in which the migrating eye had reached the sagittal plane of the head swam in an upright position, though they came to rest more often on the future eyeless side. *Within three days* after the capture of a fish in this stage both the orientation in swimming and the position of the eyes became essentially that of the adult.

The growth of the fish after turning is rapid. A sand-dab measuring 10 mm. in length and 5 mm. in depth (*i. e.*, the measurement taken along the dorso-ventral axis) was confined in a lamp-chimney aquarium for 11 days and then was found to measure 22 mm. in length and 12 mm. in depth. If the third dimension, the breadth or thickness of the fish, be assumed to increase in the same proportion, which is a reasonable assumption, the volume of this individual increased more than tenfold during the 11 days. The winter flounder of corresponding stages, according to my observations, does not grow quite so rapidly. It reaches a length of about 75 mm. by the end of August, when it is at most 7 months old.

There are six species of flatfishes comparatively common at Wood's Hole, according to Smith ('98). Three of these, Pseudopleuronectes americanus, Limanda ferruginea, and Achirus fasciatus, are dextral (*i. e.*, the fish lies normally with the right side uppermost), and three, Paralichthys dentatus, Paralichthys oblongus, and Bothus maculatus are sinistral.

Of these six species, Paralichthys dentatus probably breeds in the open sea, as small fish are not found. Paralichthys oblongus and Bothus breed in May and the sole about the end of June. I can find no account of the breeding time of Limanda. P. americanus breeds from the middle of February to the first week of April.

In the summer of 1899, when P. americanus was especially plenty, metamorphosed fish of two different lengths were taken in the tow. These were about equally abundant. The smaller measured not over 8–9 mm. at the end of metamorphosis. The larger was a more bulky fish with slightly more pigment and it was found swimming upright until it reached a length of 13–14 mm., when it also turned left side down. I found no specimen intermediate between the two lengths. The larger, more pigmented specimens may have been either the larvæ of the black-bellied variety or possibly the young of Limanda. The more important specific differences between Limanda and Pseudopleuronectes are the following: The anterior part of the lateral line of Limanda is more arched and this species has more fin-rays in both dorsal and ventral fins. But it is difficult in the young fishes to establish a satisfactory division on the basis of the number of fin-rays. According to Bumpus ('98), P. americanus at Wood's Hole averages 66.1 fin-rays to the dorsal and 49.6 to the ventral fin. Jordan and Evermann ('96–00) give for Limanda 85 dorsal and 62 ventral fin-rays. The specimens of Limanda I have counted at Wood's Hole vary from 81 to 78 in the dorsal and 61 to 47 in the ventral. I counted the fin-rays in six small fishes, three of each type, and found that in two of these — they belonged to the 14 mm. type — the rays corresponded to the formula for Limanda, and that in one (9 mm. long) they agreed with P. americanus, there being 64 dorsal and 47 ventral rays. The number of rays in the other three were absolutely intermediate, two (8.5 mm. long) having respectively 71–54 and 76–51 rays, the remaining one 75–56 rays.

The work of Kyle ('98) at the St. Andrews laboratory is valuable for comparison at this point. There are five dextral flounders on the Scotch coast which may be confused with one another. The ones most like our species are Pleuronectes flesus, the flounder, P. platessa, the plaice, and P. limanda, the dab. Of these, when metamorphosis is completed, the flounder is the shortest (about 8 mm., according to Petersen), the plaice next and the dab the longest. The plaice may vary in length from 13 to 16 mm.; the dab from 16 to 19 mm. at metamorphosis. In Danish waters (Petersen, '94, p. 14) the metamorphoses of these two species are complete when the fish is from 4 to 6 mm. shorter.

As the plaice and dab overlap each other in length, their fin formulæ were ascertained by Kyle in the hope of finding there a distinctive character. These also overlap, the dorsals varying in both forms from 68 to 77 and the anals from 50 to 61, the dab usually presenting the higher number. The flounder has from 58 to 64 dorsal rays and from 38 to 46 anal rays.

Pseudopleuronectes is intermediate in the number of fin rays between P. flesus and P. platessa. It also turns at an intermediate length. Taking Petersen's figures for Denmark, P. flesus turns at 8 mm. and P. platessa at from 10 to 11 mm. The length at which my shorter larvæ turned was from 8 to 9 mm. No individuals longer than this were found metamorphosing until the length of about 14 mm. was reached.

Limanda ferruginea has more fin-rays than P. limanda. If I am correct in the assumption that the larger, more bulky fish, which turns at a length of 14 to 15 mm., is the young of Limanda, its length at metamorphosis would be intermediate between those found for P. limanda by Kyle and by Petersen.

If this fish is the young of Limanda, another problem would be solved. How is it that, with two such distinct sizes at metamorphosis, the small flatfishes seined a month later are about uniform in size? Limanda is a comparatively deep-water fish, being found in the deepest parts only of Vineyard Sound; the young may have returned by the last of July to the region where the adults live, so that there would be left only the young of the on-shore species, P. americanus.

That I took only a few specimens of these problematical coarser larvæ in June, 1898, and that half the larvæ taken in the same month of the next year were of this kind, leads me to believe that the breeding seasons of P. americanus and Limanda may not always exactly coincide. This question can very easily be settled by breeding the fish, and satisfactorily only in that way. It may be that the pheuomena we have to deal with here are explainable in another way. Looss ('89) found that tadpoles metamorphosed in "waves," a part only of a brood changing at a time. There might be something of this sort here, metamorphosis at the one length or at the other depending on the advancement of development.

I wish to thank Mr. Alexander Agassiz for the privilege of occupying one of the Museum tables at the U. S. F. C. laboratory during parts of the summers of 1898 and 1899, and Mr. W. A. Willard for a number of brains of adult fishes. The work on the nervous anatomy was done, in part, under the direction of Dr. G. H. Parker. I am deeply indebted to Dr. E. L. Mark, at whose suggestion the work was undertaken, for useful advice and the supervision of the whole work.

III. Methods.

The killing fluids used were (1) 10% formol, (2) Flemming's stronger fluid, (3) Vom Rath's picro-sublimate mixture, (4) bichromate of potassium, (5) Gilson's fluid, arranged in the order of their value. I failed to get successful preparations with Vom Rath's platinic chloride mixture. Where decalcification was necessary Flemming's mixture gave very good results. The usual methods of further procedure for sections by the paraffin process were used. Heidenhain's iron hematoxylin gave the best stain, though Delafield's and Ehrlich's hæmatoxylins also gave successful preparations. These were followed by Congo red or acid fuchsin to differentiate fibre tracts. The acid fuchsin has the further advantage that it stains developing bone and fibrous connective tissue. The Weigert stain with copper and the Weigert-Pal method were both used in nerve study. Both adult brains and the larvæ proved to be refractory material for the Golgi method. The rapid method was used, but not more than 5 per cent of the specimens gave any impregnation whatever. A sojourn of three days in the Golgi fluid and more than two in the silver bath were found to give the most successful preparations. Material was left in the silver until wanted for sectioning, though much of it was sectioned after an exposure of two days to the silver nitrate.

IV. Migration of the Eye and Changes in the Cartilaginous Skull.

Before proceeding to describe the conditions which I have found in Pseudopleuronectes americanus, I shall give a brief account of the main results reached by previous observers, omitting for the present those of Pfeffer.

1. Summary of Previous Studies on the Migration of the Eye.

It was suggested about the middle of the last century, that the Pleuronectidæ, though unsymmetrical as adults, are, in their young stages, bilateral animals like other fish. The brief accounts of Van Beneden ('53) and Malm ('54), who found young fish quite similar in markings to adult flatfishes, but with eyes in a different position, seemed to indicate the possibility that one of the eyes migrated around the head from one side to the other.

The first paper which really describes a method of transition of the eye in flatfishes is that of Steenstrup ('63). According to Wyville Thomson ('65), on whose abstract of Steenstrup's paper I have relied (see also Steenstrup, '64), this author contends that the final position of the eyes cannot be explained as simply the result of a torsion of the front part of the head; and there is, in his (S.'s) opinion, a penetration of the tissues of the head by one of the eyes. This process Steenstrup described carefully from alcoholic specimens of different sizes of the young forms which he provisionally termed Plagusiæ. In this species development resulted in a sinistral flounder, *i. e.*, one in which the left side during adult life is uppermost. The right eye was slightly in advance of, as well as dorsal to, the left eye. The mouth became oblique toward the blind side, and the posterior part of the face, where the normal eye is located, seemed pressed "upward" toward the future eye-side. The right eye no longer projected from its own side of the head in a large orbit, but was deeply imbedded in the tissues, so that it had only a small orbit-opening on the right side. Later, an opening was made on the left side and for a time the eye had two orbits. The original orbit soon closed, and as the eye reached the surface level on the left side of the head the new orbit increased in size. This second orbit was described by Thomson as a bony one in the adult fish, being formed, so Thomson contended, by the frontal and prefrontal of both sides.

Schiödte ('68), working on other species, showed that the passage of the eye around the head is a normal method of development. The penetration of the eye through the tissues of the head is restricted to a few fishes whose larval forms were once considered adults, and given the name Plagusia.

He observed a Pleuronectes platessa — a dextral flounder — 10 millimetres long, of which he says, "The right eye stands over the beginning of the lower third of the maxillary bone. The left eye stands at the top of the head, so much inclined to the right that from the left side only slightly more than one-third of the pupil can be seen; it stands in front of the dorsal fin, so that the latter is just behind the end of the left and [the] beginning of the middle thirds of the eye." In a 14 mm. specimen the pupil of the left eye had become invisible from the left side and the dorsal fin touched the left margin of this eye, the foremost ray being a little in advance of the extreme posterior margin of the eye. In a 40 mm. fish the right eye had moved so that it stood over the lower end of its maxillary bone and the left eye had followed it, so that they were almost as close to each other as in the last stage, the left eye being a little farther back than the right. In this specimen the dorsal fin reached as far forward as the middle of the left eye.

Schiödte held from these observations that the dorsal fin kept its position and that the left eye migrated forward around it and then passed backward to its final position. His implied argument, if I understand him rightly, is, that the right eye moves backward from a position over the lower (posterior) third of the maxillary bone to one over its lower (posterior) extremity, and that the left eye moves backward still further proportionally, because in the end (the 40 mm. specimen) it is not only above but "a little behind" the right eye. This conclusion was in his opinion confirmed by the observation that the rays in the dorsal fin of young specimens corresponded in number with those of the adult.

He described under the name Bascanius tædifer, n. s., a peculiar flounder (evidently sinistral), which had a semilunar depression between the right eye and dorsal fin. Here the body was so thin that, *if incautiously handled*, it broke in pieces or separated itself from the dorsal fin. In that case a part of the right eye appeared through the hole, giving the animal the appearance of possessing two eyes and a half.

Agassiz ('78) described definitely for the first time the two methods of development by which the eyes of flatfishes change position. His description of the method by migration *around* the head is briefly as follows (p. 5): "The first change — and the process is identical, whether we take a dextral or sinistral flounder — is the slight advance toward the snout of the eye about to be transferred. . . . This movement of translation is soon followed by a slight movement of rotation; so that, when the young fish is seen in profile, the eyes of the two sides no longer appear in the same plane, — that on the blind side being slightly above and in advance of that on the [future] colored side. With increasing age, the eye on the blind side rises higher and higher toward the median longitudinal line of the head; a larger and larger part of this eye becoming visible from the colored side where the embryo is seen in profile, until the eye of the blind side has, for all practical purposes, passed over to the colored side."

Later the dorsal fin finds its way forward toward the nose, dorsal to the transposed eye.

Agassiz also well described the method by penetration discovered by Steenstrup in Plagusia. The change was followed day by day in fishes kept captive in his Newport laboratory. He pointed out that these two methods are merely two extremes of the same process; probably the peculiar fish described by Schiödte was an example of an intermediate method.

Only two other descriptions of intermediate methods of eye-transition need be noticed. Ehrenbaum ('96) has discussed, among other points, metamorphosis in the flatfishes of the German Ocean. Stages of the larvæ of the commoner species in which the eye passes around the head are given. In the larva of Arnoglossus laterna, which strongly resembles the so-called Plagusiæ, the dorsal fin extends to the nostril while the fish is yet symmetrical, so that the eye *must* pass under the dorsal fin as in Plagusia. The prolongation of the dorsal fin to the nasal pit and the position of the right eye close to the lower margin of the fin (after migration) prove, in Ehrenbaum's opinion, that the right eye is shoved through *under the dorsal fin* from the right to the left side.

Recently a Japanese zoölogist, T. Nishikawa ('97), found a case where the dorsal fin extended along the head as far as the end of the snout in close contact with, but not fused to, the skin. There were no fin rays located in the eye region. The right eye passed through a slit between the fin and the head in one day, passing thus from one side completely to the other. Unfortunately the fish died, so that it is not known whether the fin would have fused later to the dorsal part of the head or not.

2. DESCRIPTION OF STAGES.

For convenience of description four stages of development may be recognized in Pseudopleuronectes americanus.

Stage I., the recently hatched fish, is represented (Plate 1, Fig. 1) by a specimen 3.5 mm. long and 12 days old. Owing to its wide dorsal and ventral fins being so transparent as to be scarcely visible, the living animal resembles, in its general appearance, a very minute pin with an elongated head. It is essentially symmetrical. I have sectioned the eggs as well as the young fish and find a close resemblance to the figures given by Fullarton ('91) in his work on the development of the plaice, Pleuronectes platessa, which is the nearest European representative of our flatfish. His drawings, too, show the eyes to be symmetrical in position. There are few pigment cells in the body of an animal of this stage and they are arranged in much broken longitudinal lines.

The largest of the recently hatched fishes are nearly as long as the smallest of the pelagic larvæ (Stage II., Plate 1, Fig. 3), which were taken the first of June; but between the two there is a great difference in depth and bulk. To this stage are assigned all those fishes which, in a strictly lateral view from either side, exhibit only one eye. The shorter, proportionately deeper, larvæ metamorphose when they reach 8 or 9 mm. in length. The degree of symmetry can better be seen in a front view (Fig. 4) of a fish 4 mm. long, the only trace of asymmetry at this stage being the slight elevation of the left nasal pit and the lack of absolute bilateral symmetry in the shape of the mouth. The upper lip is slightly drawn upward on the right side directly opposite the right nasal pit (*fv. olf.*).

Stage III. (Fig. 2) has been made to include those fishes in which the eye of the blind side had so far migrated as to be visible when the fish was viewed in profile from the ocular side. At this stage the eye lies in the median plane in a depression immediately in front of the dorsal fin, which has grown forward since the preceding stage. There is also a noticeable change in the direction of the urostyle[1] (*ur'stl.*).

In the last stage, IV., the eye has completed its migration, and, so far as regards the distortion of the head, the fish is essentially in the adult condition. Changes after this are merely accentuations of what is found here. Figure 6 shows the dorsal fin (*pin. d.*) at this stage extending as far forward as the middle of the eye. On the body are to be seen the beginnings of the pigment areas which later color the right side of the fish.

The sinistral fish, Bothus, is at first symmetrically pigmented. The lower side does not become colorless until the disappearance of the first color pattern and the establishment of the much lighter adolescent color, which comes after the turning. P. americanus, on the contrary, is essentially non-pigmented until it is ready to become a bottom feeder.

The front view of P. americanus at this stage (Fig. 5) — the completely turned fish — is most instructive in bringing out the want of symmetry. The left eye has moved through an arc of about 115 degrees, as may be seen by comparing this view with that of Stage II. (Fig. 4). The left nostril has moved dextrad and dorsad, as if in the passage of the eye it, too, had become involved. The angle of the mouth on the right side bends sharply ventrad; and the upper lip of the right side is apparently drawn dorsad toward the right nasal pit. From this point the mouth opening has the form of a long slit which extends to the left and ventrad in a nearly straight line.

In Paralichthys oblongus and in Bothus the mouth remains nearly horizontal and symmetrical.

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[1] For the development of the caudal fin of the flounder, see Agassiz ('78).

3. Homologies of the Anterior Bones of the Skull.

The changes in the cartilaginous facial skeleton will be more easily set before the reader, if the homologies of the bones of the face as explained by the more recent writers be first made clear.

The papers of Pfeffer (**'86**, **'94**), which deal with the cartilaginous skeleton, are also reviewed here.

Traquair (**'65**) has given a careful account of the adult skulls of flounders of both dextral and sinistral types. The greatest changes, as compared with a symmetrical fish, the cod, he finds in the facial region; the brain case remaining nearly symmetrical, except with regard to the position of the ridges and wings on the bodies of the bones for the attachment of muscles.

The adult skulls of (1) the halibut, (2) the pole flounder, and (3) the plaice (Platessa vulgaris) form a series, in which he shows that there is a progressive modification, especially of the frontal bones. In the halibut, though the main part of the frontal of the "eyeless" side is back of the migrating eye, a thin curved process from it extends between the two eyes and with the corresponding interocular process of the frontal of the ocular side (to which it is closely applied) forms a part of the orbit of the migrating eye. In the case of the pole flounder this process from the frontal of the eyeless side is reduced to an exceedingly thin curved strip. Finally, in the common flounder even this thin strip has entirely disappeared, so that the frontal of the eyeless side is now joined with the front of the head exclusively by means of the great external connection, since called by German writers the "Brücke."[1]

Steenstrup (**'63**), according to Thomson (**'65**), considered the "Brücke" the principal frontal of the eyeless side.

Thomson himself thought that it represented the prefrontal of the eyeless side, and that the partition between the eyes was the frontal of the ocular side.

Malm (**'68**) at first held the "Brücke" to be infraorbital, but later adopted Steenstrup's view.

Reichert (**'74**), disregarding the beliefs of previous authors, decided that the frontal formed two infraorbital processes, which then fused with the latent "Brücke" to form the orbital ring. The parts between the eyes he thought were normal.

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[1] This is a new and peculiar bridge or bar (pseudomesial) of bone which has no (single) equivalent in the crania of symmetrical fishes.

Klein ('68) called the outer edge of the "Brücke" prefrontal, and the inner and hinder part of the same, principal frontal.

Traquair ('65, pp. 276, 277) summarizes the changes from the condition of the symmetrical type of skull as follows:

"(1) The mesial vertical plane of the cranium has become inclined over to the now binocular side, very slightly in the posterior part of the cranium, very much in the region of the eyes (so that the original vertical interorbital septum becomes now nearly horizontal), returning in the nasal region nearly to its original vertical position in the turbot, but never doing so in the halibut or plaice.

"(2) In consequence of this, the middle line of the base of the skull remains still comparatively straight; while the middle line of the upper surface, diverging from the apparent or pseudomesial line, curves round between the eyes, . . . and returns to the middle in front. Having got in front of the eyes and nasal fossæ in the turbot, it again coincides, or nearly so, with the apparent middle line; but in the halibut, and still more in the plaice, the apparent and morphological middle lines, if produced, would cross each other.

"(3) In the anterior part of the cranium, the parts on the eyeless side of the middle line of the *base* are, in all the Pleuronectidæ, more developed than on the ocular side. . . .

"(4) On the top of the head the interocular parts of the frontal and prefrontal bones are more developed on the ocular side. The interocular process of the frontal of the ocular side is always much stouter than that of the other [eyeless side] bone, and always articulates with a corresponding process sent back from the prefrontal. But the prefrontal of the eyeless side sends back no process to articulate with the frontal of the same side, whose interocular part, if examined in a series of flatfishes, gets smaller and smaller, till in the plaice it seems almost gone. The same condition affects the morphologically mesial plate of cartilage forming the anterior part of the interocular septum, which cartilage we have already seen to be chiefly developed on the ocular side.

"(5) To accommodate the two eyes, now both on one side of the head, the anterior parts of the frontal bones remain as a narrow bar, never widening out into a broad arch as in the cod and other fishes. Accordingly, to maintain the requisite stability of the cranium, a new bar or bridge of bone is formed (pseudomesial) by the union of a process sent forwards from the anterior external angle of the frontal of the eyeless side with one sent back from the corresponding prefrontal. By means of this bar the upper eye becomes closed round by a bony orbit, whose boundaries in the turbot consist of the interocular process of the frontal of the eyeless side, the external angular process of the same bone, the external angular process of the corresponding prefrontal, and a small portion of cartilage in front. In the halibut and plaice, however, the nasal bone comes to take part in the boundary of the orbit principally by a development from its eyeless side; and in the latter fish, owing to the atrophy of the interocular portion of the frontal of the eyeless side, the corresponding part of the other frontal forms almost the entire external boundary of the orbit.

"(6) The olfactory foramen and the place of suspension of the anterior suborbital bone are further forward on the ocular side. . . . The articulation of the epitympanic bone to the cranium, in the halibut and plaice, likewise extends further forward on the ocular side.

"(7) The axis of the keel of the cranium . . . points . . . to the eyeless side."

Pfeffer in a preliminary paper ('86) without illustrations, has described the larval stages of development in one of the Pleuronectidae. As he is the only writer who speaks of the conditions in the interior of the head, his conclusions are given in some detail.

The young fish has an entirely cartilaginous cranium, in which the eye sockets are separated below by the sphenoid, and above by the interorbital roof (Zwischenaugen-Decke); but between these the sockets communicate freely with each other. The ethmoid, constituting the anterior part of the cranium, develops a wing on each side, the place where the wings join the body of the ethmoid being marked by the presence of the nasal openings. In very young animals the bulbi olfactorii are embraced by the ethmoidal roof; but later they are forced backward behind it.

Over the interorbital and ethmoidal regions runs a ridge-like dermal bone, which is triangular in cross section, and stands vertically; it supports the dorsal fin, and is at first free from the cranium. It is the "principal frontal" of authors.

In the second stage examined by Pfeffer, the migratory eye has risen so that half of it is above the level of the interorbital roof. The brain capsule remains unchanged, except that it has received the bulbus olfactorius, which has been forced backward by the migration of the eye. The interorbital roof is bent outward toward the eye side and somewhat twisted on its long axis. At the same time the frontal, now grown fast to the interorbital, makes with it a great bend. However, only a broad band — its basal portion — remains, while the greater, vertical part of it is for the most part resorbed by the migrating eye. There now remains between the migrating eye and the eye side only the translucent, thin outer skin which previously covered the dermal bone. The front part of the ethmoidal region is symmetrical; but the upper part of the wing of the eye side has fused to the fronto-orbital and is now continuous with the developing supraorbital cartilage [bone?], while the whole rim of the wing of the blind side remains free.

The transposed eye at a later stage occupies a pit which opens upward and toward the eye side and is surrounded by a high rim of thin dermal bones. The previously upper side of the eye now lies on the interorbital septum, therefore most ventral; whereas the previously lower side of the eye is now near the dorsal fin, therefore highest. The eye has thus rotated 180 degrees. The side of the migrating eye that is turned toward the blind side of the head is now closed in by the formation of new dermal bones. The socket is completely open in the region of the optic nerve. By the migration of the eye, the anterior oblique eye muscles, which arise from the hinder border of the ethmoid, are laid bare; a thin covering of dermal bone grows over these also. The wing of the ethmoid on the eyeless side, is fused to a part homologous with the supraorbital cartilages; these grow upward and inward, the latter helps in forming the anterior wall of the new orbit.

Pfeffer says that, though the ossification is a continuous process, one may distinguish, if he will, three stages in the development of the parostotic cranial bones of fishes, characterized by —

(1) The first delicate osseous investment of the cartilage;

(2) The dermal ossification which establishes approximately the permanent forms of the bone;

(3) The ridges, crests, wings, and the like, — entirely superficial additions, — which are probably always connected with muscular action.

In the flounder the rotation begins while the frontal region of the young fish is in the first of these stages. Soon the frontal (cartilaginous) is in quite another place, under quite another region of the skin. When it has changed its position, there is dermal bone produced over it in its new position; but there is not the least reason why the skin under which it would normally have lain should suddenly lose the power of producing bone, — and in fact it does not, for it produces the bridge. The bony bridge, then, is the parostotic ossification of a precise region of the cutis, and if the cranium had remained symmetrical, it would have fused to the frontal; but inasmuch as there is a displacement of the region of the (cartilaginous) skull, this dermal ossification has become attached to those bones which took a position directly beneath this bone-producing region of the cutis after the displacement of the (cartilaginous) skull.

Pfeffer's final paper, so far as I know, has not yet appeared; but in a short note ('94) the author states again that the interorbital septum twists on its long axis, and adds: (1) that the migrating eye, when it reaches the mid-line, loses the thin patch of skin which has separated the cornea from the outer world, and (2) that the dorsal fin, the muscles and the bones develop along the physiological axis of the body, the continuation of the spinal column.

4. CHANGES IN THE CARTILAGINOUS SKULL.

In order to have freshly in mind the normal condition of the cartilaginous skull in fishes with which to compare the youngest flounder skulls, I give a brief statement of the essential parts of Parker's ('73) paper on the skull of the salmon:

In a salmon of the second week, according to Parker, the cartilaginous skeleton is fully formed. There is a large fossa on the top of the head over the mid-brain. In front, the skull is roofed over with a thin cartilaginous plate, the ethmoidal "tentorium," or tegmen cranii. Anteriorly this is directly continuous with the ethmoid; its posterior lateral corners are connected with the cartilage of the auditory region by the supraorbital bars, which curve upward and outward. The ethmoid is continuous with the trabeculæ cranii, — now fused together in front, but diverging behind, — which run backward forming a partial floor to the skull cavity. The superior and inferior oblique eye muscles have their origin on the posterior face of the ethmoid. The recti originate from a lamina on the hinder part of the parasphenoid.

I have projected upon the frontal plane the cartilages of the facial region of Pseudopleuronectes in each of the four stages. But because of the great length of the dorso-ventral axis of the older stages, this method needs to be supplemented either by projections upon the sagittal plane or by some other process. The most satisfactory reconstruction is, of course, the model. Accordingly with the aid of sections I have modelled in wax by Born's method the facial region of Stages II., III., and IV., and cuts made from photographs of these models are given in the text.

a. *Stage I.*

A dorsal view of the cartilages of the facial region in Stage I. is shown in Figure 7 (Plate 1) as they appear in frontal projection. As in the salmon (Parker, '73), the first cartilages to form are the trabeculæ cranii and Meckel's cartilage. The slight want of uniformity in the shape of Meckel's cartilage on the two sides may be merely an individual variation. Certainly this cartilage is essentially symmetrical. The line passing through the middle (third) brain ventricle and between the lobes of the tectum and cerebrum I have assumed to lie in the sagittal plane in a normal fish of this stage. This plane, represented in projection in the figure by the two ends of a fine line, cuts lengthwise the fused trabeculæ, dividing the mass at the anterior end, which is to be the future ethmoid, nearly into halves. The line falls midway between the two arms of the trabeculæ, where they diverge to allow space for the pituitary body. In front the ethmoidal mass overlaps slightly, on either side, Meckel's cartilage a little behind its points of sharpest curvature.

In the flatfishes there is no distinct "tentorium," or tegmen cranii, extending backward from the ethmoid to roof over the front part of the brain case, as there is in the salmon.

b. *Stage II.*

Between Stages I. and II. there is an interval of six weeks and the manner of differentiation of the many cartilages and projections found in Stage II. (Fig. *A* and Plate **2**, Fig. 10) cannot be traced here. Figure 10 is a dorsal view of the facial cartilages of this stage. But, as it gives a less complete view than the model of the same specimen (Fig. *A*), I call attention to the two supraorbital bars only — the complete one on the right (*trb. su'orb. dx.*), fastened to the right ethmoid wing, and the two parts (*a.* and *p.*) of the left one, between which is

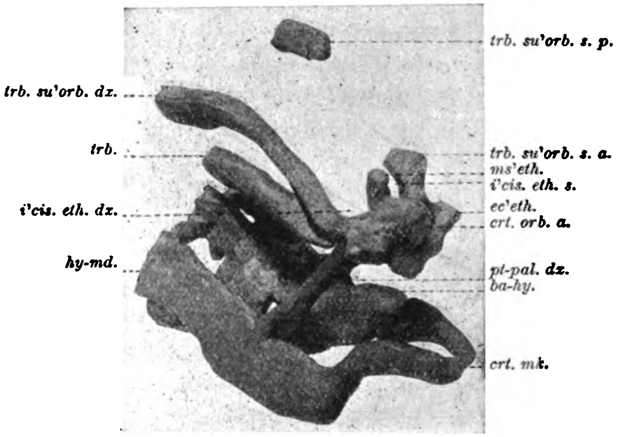

FIG. *A*.

Oblique view of the facial cartilages of *P. americanus*, Stage II. Photographed from a wax model (Born's method) seen from a point midway between sagittal and transverse planes and about 30° above the horizontal plane. × 75. For meaning of lettering, see Abbreviations under Explanation of Plates.

the space through which later the eye must pass. Figure *A* is from a photograph of the model of the front part of the cartilaginous cranium of a 3.5 mm. fish, viewed obliquely from the front, the right side, and above. The line of vision makes an angle of about 30 degrees with the horizontal plane. *Meckel's cartilage* no longer forms a simple bow lying in the horizontal plane. The anterior end is curved slightly ventrad, and the bar of either side in passing backwards bends sharply ventrad to join, nearly at right angles, a series of cartilaginous masses (Fig. *A hy-md.*) representing the future quadrate, articular, symplectic, and hyomandibular bones. In cross section these cartilaginous masses have, in general, the form of an elongated oval, the axis of which inclines dorsad and mesiad; the ventral margin is slightly thicker than the upper. The space occupied by each separate cartilage in this series is not indicated in the models, though in the sections the boundaries can be determined by the presence of the connective-tissue sheaths which limit the cartilages.

The *pterygo-palatine* bars (*pt-pal.*) extend ventrad and caudad from each side of the ethmoid to the quadrate region (compare also Fig. 10). At this stage the fish has a very small gape. The hyoid and gill-arch cartilages are present in their general shape, occupying most of the space between the right and left hyomandibular-quadrate masses, and ending in front just beneath the body of the ethmoid in the basi-hyal (*ba-hy*).

From the ethmoid mass arise also the *supraorbital bars.* These, in the salmon, extend backward from the ethmoid, curving upward and outward above the eyes, to the heavy cartilaginous mass of the otic capsules. In the flatfish of this stage, as shown in the reconstruction, there is but one complete supraorbital bar (the right), the left being represented by two remnants, an anterior and a posterior; the anterior (*trb. su'orb. s. a.*) is a process extending backward from the dorsal left-hand corner of the ethmoid; the posterior (*trb. su'orb. s. p.*) extends forward from the left otic capsule. It is through the space between these two projections that the left eye migrates. While, as yet, there is no external sign of an asymmetrical position of the eyes, internally preparations for such a condition are clearly established, for the middle portion of the left supraorbital bar has disappeared.

I have sectioned only a few individuals of P. americanus in which the left supraorbital bar is still continuous, and even in them at the region corresponding to a transverse plane passing through the middle of the two eyes the bar is so reduced in thickness as to show in cross section only one or two cartilage cells.

Since Bothus spawns in May, I was able to get specimens which were certainly not more than one month old. The one shown in frontal section in Figure 14 (Plate 3) was 2 mm. long. However, as P. americanus grows much more slowly than Bothus, it is not possible to compare ages on the basis of relative lengths. In Bothus at this stage both supraorbital bars are present and there is as yet no sign of reduction in either of them. In the sinistral flounder (Bothus) it is, of course, the right supraorbital bar which disappears to give passage for the eye, whereas in P. americanus it is the left. Since in the middle of the bar its plane slants inward and downward, and since the bar in its course from ear capsule to ethmoid is also slightly convex dorsally, it is evident that no one section in any plane could show the whole bar. Both bars extend over the eyes, as can be seen from the position of the dotted lines shown in the figure (Plate 3, Fig. 14), which represent the location of the eyes, as seen in a more ventral section, accurately projected upon the plane of this section.

Appearances of degeneration in P. americanus taken after June 1 are rare. The youngest fish must be at least six weeks old at that time, and only the most nearly symmetrical of the smallest fishes sectioned show any trace of the left supraorbital bar, either normal or degenerating. Figure 15 (Plate 3) shows the appearance, in frontal section, of the anterior degenerating end of the posterior remnant in P. americanus at Stage III. *a*, extending forward from the region of the ear capsule. The whole section of the bar has been drawn, so as to show the difference in appearances at the two ends. The cell bodies (*cl. crt.*) at the anterior end of the bar are much shrunken and the intercellular ground substance has for the most part disappeared. The nuclei are much crowded, have lost the characteristic form seen in most normal nuclei, and are angular and dense in appearance.

The degenerating portion of the cartilage is darker than the unchanged cartilage cells next to it. The connective-tissue sheath (*tu. co'nt. tis.*) around the cartilage is, however, persistent and can be traced to the ethmoid.

In this specimen there is a coagulum filling the space in which the degenerated portion of the cartilage bar formerly lay. The presence of this coagulum is easily accounted for on the assumption that the sheath has retained the material resulting from the degeneration of the cartilage cells, and that the killing fluid has caused it to be precipitated. This condition is similar to that observed by Looss ('89) in the resorption of cartilage in the tail of the tadpole. In that case, according to Looss's interpretation, it was the chorda sheath which restricted the diffusion of some of the products of the degenerating cells. He, too, found that the intercellular substance was the first to disappear in resorption.

Whether the cartilage nuclei, when set free by the disintegration of the intercellular substance, degenerate completely, or join the nuclei of the connective tissue, I cannot determine. There is much resemblance between the compact nuclei of degenerating cells and those of the sheath.

Since the bar disappears first in the middle region, there are, for a short time, two degenerating regions, one which will end at the ethmoid and the other at the persistent stub in front of the ear capsule. The location of these will be evident by reference to Plate 2, Figure 10 (*trb. su'orb. s. a.* and *p.*).

When in P. americanus the frontal of the eyeless side is formed, its main body takes the position of this posterior stump of the left supraorbital bar. It is significant that there is no more space provided by this degeneration than is barely necessary for the ready passage of the eye.

The body of the ethmoid is very irregular in shape. Besides the two wings with which the supraorbitals are connected, there is a median elevation in the sagittal plane of the fish (*ms'eth.*, Fig. *A*), and a forward knob-like projection (*crt. orb. a.*) in the same plane. The two olfactory pits lie just in front of the wings of the ethmoid, and the olfactory nerves pass to them through the two deep notches (*i'cis. eth. dx.* and *s.*) seen on the dorsal surface of the cartilage. The right nerve passes between the supraorbital bar of the right side and the median elevation; the left nerve between the left supraorbital stub and the median elevation. In this left notch the superior oblique muscle of the left eye takes its origin, and in some cases the superior oblique muscle of the right eye has its origin also close to that of the left eye, therefore at the left of the sagittal plane.

c. *Stage III a.*

Figure *B* is photographed from the model of the cartilages of a fish of Stage III. (Plate 1, Fig. 2), where the left eye could be barely seen projecting over the top of the head as the fish lay on its left side. The left wing of the ethmoid cartilage (*ec'eth. s.*) has no longer any trace of the projection representing the anterior portion of the left supraorbital bar. The posterior portion of the bar (*trb. su'orb. s. p.*) projects forward from the ear capsule substantially as in Stage II., there being just room for the eye — now, of course, increased in size — to pass between the front end of it and the ethmoid. The right supraorbital becomes a little more arched as the fish increases in depth. The wings of the ethmoid extend out from the mid-line farther proportionally and are more flattened antero-posteriorly. Upon the surface of these wings of the ethmoid cartilage the ect-ethmoid bones, or pre-frontals, are later formed.

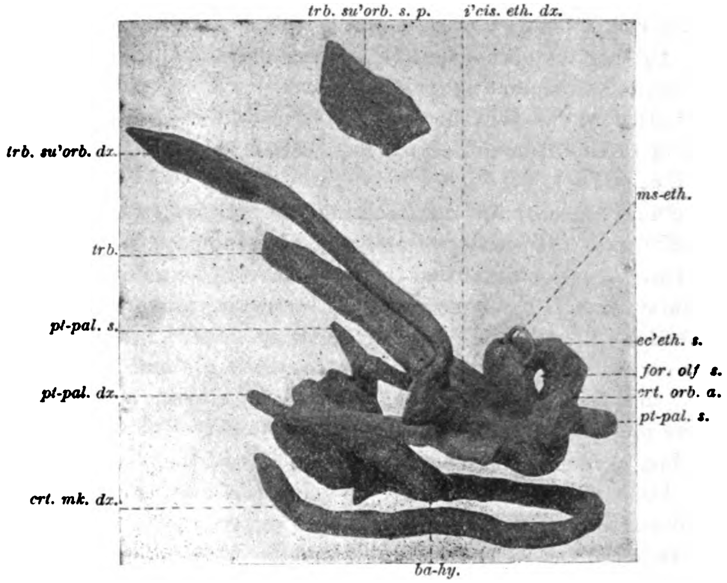

Fig. *B.*

Oblique view of the facial cartilages of *P. americanus*, Stage III. Photographed from a model, as in the case of Fig. *A.* × circa 75.
For meaning of lettering, see Abbreviations under Explanation of Plates.

The gape has been greatly increased by the growth in length of all the facial cartilages, but these have not increased in diameter proportionately. The pterygo-palatine bars, which from the first support the upper jaw, in lengthening have come to lie nearly parallel to Meckel's cartilage, and their articulation with the quadrates is so far posterior that the one of the left side alone falls within the region modelled. At this stage these cartilages are in some instances so reduced in diameter toward their posterior ends, as to show in cross sections only one cartilage cell. A process from the left wing of the ethmoid has fused with the median region of the ethmoid, thus bridging over the left ethmoid notch and leaving between the mes-ethmoid and the region of the anterior end of the right supraorbital cartilage an orifice (*for. olf. s.*), which corresponds to the notch on the right. In other specimens I find that both wings of the ethmoid have sent out processes to fuse with the mes-ethmoid, thus converting both notches into foramina for the passage of the olfactory nerves to their capsules on the front of the ethmoid.

In this model a bent wire is inserted into the mes-ethmoid in the median plane to aid in locating the position of that plane, — the plane in which the future interorbital septum is to develop. There is as yet no trace of this septum in the specimen modelled; but Figure 18 (Plate 4) shows a cross section of the head of a fish (P. americanus) of this stage, which does indicate the position of the future interorbital septum. The fine vertical lines outside the figure represent the projection of the sagittal plane of the fish. A small bar of cartilage (*arc. eth. m.*) is seen in cross section above the mes-ethmoid. Traced anteriorly a few sections, this fuses with the ethmoid. Traced posteriorly it soon unites with the thin fused trabeculæ cranii not far from where they pass over into the ethmoid. It is, then, a slanting bar, or arch, from near the anterior end of the trabeculæ cranii to the posterior face of the ethmoid. In another specimen (Figure *C′*, p. 24) this arch has become larger and appears as the forward prolongation of the trabeculæ (*trb.*). In the space beneath this arch lie the oblique eye muscles, two of which (the right and left inferior oblique) appear in Figure 18. The same figure shows that the migrating eye may exert pressure directly on the cartilage, for the left eye-ball is indented by the left wing of the ethmoid.

In another specimen of this stage, which had lost the migrating eye in the process of turning, there were certain peculiarities worthy of consideration. This fish, too, had a well-developed median arched cartilage on the posterior face of the ethmoid. The right superior oblique muscle had its origin at the angle produced by the junction of the arch and the body of the ethmoid. The inferior oblique was attached lower, at the angle made by the union of the ethmoid and the trabeculæ. The posterior face of the ethmoid is the usual place of attachment for these muscles, though a specimen of B. maculatus had both the inferior and superior oblique muscles attached on the median arched bar. The most noticeable peculiarity of this specimen was shown in the origin of the supraorbitals. As I have said, there was no eye present on the left side. The anterior end of the left supraorbital bar still persisted in this specimen in the form of a stub projecting backward and slightly upward from the left wing of the ethmoid, though unmaimed individuals whose cartilages were otherwise in a like stage of advancement showed no traces of it. Furthermore, the stub, instead of disappearing by a gradual reduction of its diameter in the region midway between the ethmoid and the ear-capsule, through which the eye normally passes, preserved the bar-like shape — the flat side being directed towards the top of the head — until its abrupt disappearance behind the middle region of what should have been the path of the migratory eye. Both supraorbitals, instead of being backward extensions of the wings of the ethmoid, as in most other specimens examined, took their origin from a mes-ethmoid enlargement which extended backward directly above the median arch that indicates the position of the future interorbital septum. In this specimen there was, therefore, a suggestion of a tegmen cranii, such as has been described by Parker for the salmon. This, instead of being a complete roof, however, was a comparatively narrow plate of cartilage which extended backward toward the brain region.

In describing the model of Stage II., a prominence (Figure *A*, *crt. orb. a.*) on the front face of the ethmoid was mentioned. This prominence is really a separate cartilaginous mass, resting in a socket of the ethmoid. There is also a pair of small labial cartilages in front of and below this plate; but owing to their small size and the difficulty of preserving small detached processes on the wax plates, they have been omitted from the models. In Stage III. this large cartilaginous mass has become rounded and projects further forward from the body of the ethmoid. Its future history will be given in connection with the description of the most advanced stage modelled (Figure *D*).

d. *Stage III b.*

The forms of the cartilages change very rapidly at this stage of development, and it is with some difficulty that one finds a cranium exhibiting a condition intermediate between Stage III *a* (Fig. *B*) and Stage IV. (Fig. *D*), which shows the completely twisted head. However, I found one fish, larger than many of the recently metamorphosed specimens, which I have designated as Stage III *b*, to distinguish it from the more common condition just described as Stage III *a*.

In this specimen (Figs. *C* and *C′*) the left eye lies in the sagittal plane, even though the fish is 15.5 mm. long, the eye usually being transformed when the fish reaches a length of 13.5 to 14 mm. There is no trace of the left supraorbital bar. The right supraorbital (*trb.*

su'orb. dx.), as but now described for the specimen that had lost the left eye, is the backward extension of a plate of cartilage which connects the right ect-ethmoid with the median mes-ethmoid arch. This flattened anterior portion of the right supraorbital cartilage corresponds to the tegmen cranii of the right side of the head in the salmon. The median mes-ethmoid arch is, at its anterior end, fused to this plate or partial tegmen, but from the short region of fusion backward for some distance the two cartilages are merely crowded closely together, a distinct line of perichondrial connective tissue being found between them. The cartilages then diverge, as may be seen in Figure *C'*, and the median mass continues backward as the fused trabeculæ cranii, while the higher, lateral portion, the right supraorbital bar (*trb. su'orb. dx.*, Figs. *C* and *C'*), passes upward and backward to the ear capsule.

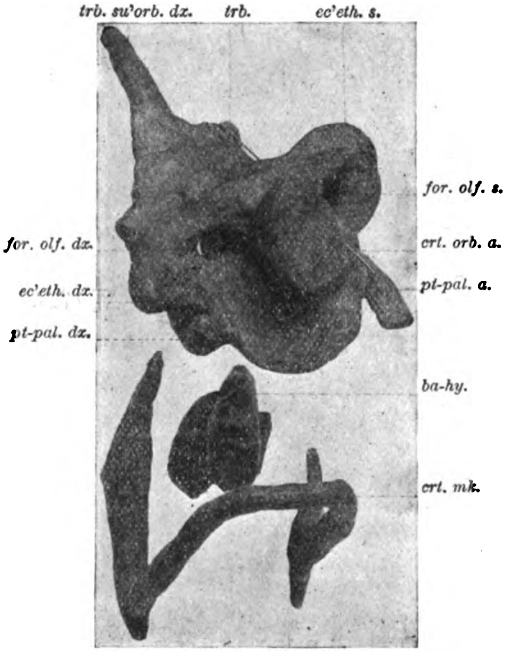

FIG. *C*.

From photograph of wax model of the facial cartilages of a large specimen of *P. americanus* intermediate between the stages shown in Fig. *B*. and Fig. *D*. Viewed from a point nearly in front, only a little to the right of the sagittal and a little above the horizontal plane. × 45.
For meaning of lettering, see Abbreviations under Explanation of Plates.

In older specimens this *right* supraorbital begins now to disappear, the disappearance progressing from behind forward as the ensheathing ocular-frontal takes its place and function. The remnant of this cartilage (*ham. eth.*), as it appears at a later stage, when it has been forced into the horizontal position (vertical as the fish lies on its side), is shown in Figure *D*. There is no longer a region of close appression without fusion between it and the median arch, but the hook arises directly from the arch.

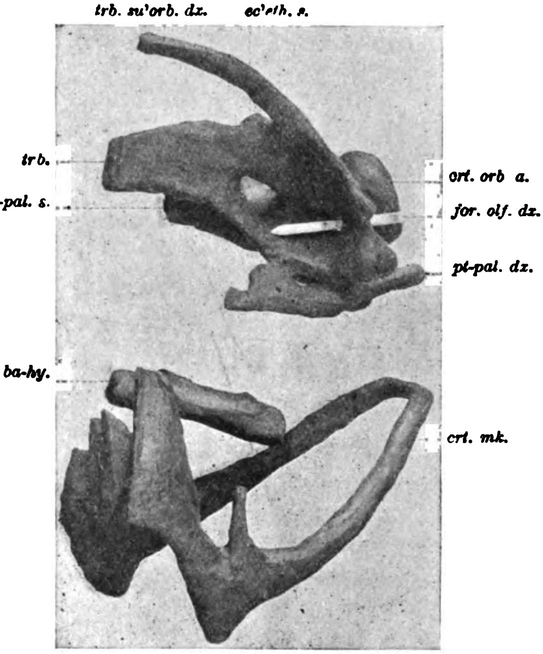

Fig. *C'*.

Same model as that shown in Fig. *C*, viewed obliquely from right side and behind. A probe is thrust through the right olfactory foramen. × 45. For meaning of lettering, see Abbreviations under Explanation of Plates.

In Figure *C* a bristle is shown passing through the left olfactory foramen, to indicate the axis of the opening, which now is not parallel to the longitudinal axis of the fish, — as the right olfactory foramen still is, — but makes with it an angle of about 45 degrees, being directed caudad, mediad, and dorsad. In Figure *C'* a white probe marks the position and direction of the right opening.

There is also indicated at this stage a beginning of the forward rotation of the dorsal margin of the ect-ethmoid cartilages about a transverse axis passing through them. The end of the bristle (Fig. *C*) over the trabeculæ cranii is, therefore, not greatly posterior to the outer end, which is seen against the left pterygo-palatine as a background. The final result of this rotation of the ect-ethmoids about the axis connecting them is to make the axes of both foramina transverse instead of longitudinal. Consequently in an oblique view from the right side, as in Figure *D*, one is looking at the olfactory foramina from that face of the ect-ethmoids which at an earlier stage (Figs. *A*, *B*) was directed posteriad. Instead, therefore, of seeing the ends of the olfactory nerves which are *distal* to the foramina, as would be the case if the cartilages were viewed from the same direction at an earlier stage (Figs. *A*, *B*, and *C*) one would now see their *proximal* ends.

A twisting of the ethmoids (in a clockwise direction when viewed from behind) about the antero-posterior axis of the fish, greater than is indicated in Figure *C*, results in the further elevation of the ect-ethmoid, olfactory foramen, and pterygo-palatine of the left side, while the supraorbital, the ect-ethmoid, the olfactory foramen and the pterygo-palatine of the right side are correspondingly depressed.

e. *Stage IV.*

The oldest facial region modelled (Fig. *D*) — that of a small fish (Plate 1, Figs. 5, 6) having the eyes in the adult position — represents my Stage IV.

The eyes are located one on each side of the flat hook-like plate of cartilage (Fig. *D*, *ham. eth.*) which, with the previously mentioned median arch (*arc. eth. m.*), runs back along the morphologically median plane (the plane between the eyes). The interorbital septum of connective tissue is continuous with these two cartilaginous processes, filling the space between them and extending thence backward. That this occupies the morphologically median plane, is proven by the position of the olfactory nerves, which lie one on each side of this septum. Anteriorly the left nerve passes through the opening (*for. olf. s.*) seen in the left (now upper) wing of the ethmoid and ends in the nasal capsule, which lies immediately in front of it. The right nerve comes from below the hook-shaped cartilage and passes through a foramen (*for. olf. dx.*) in the anterior part of the ethmoid to the right nasal capsule, which is located somewhat in front of the ethmoid and near the anterior end of the right pterygo-palatine.

The external opening of the left nasal pit is about 30° higher in Stage IV. (Fig. 5) than in Stage II. (Fig. 4).

The superior oblique muscles of the eyes have their origins at or near the junction of the median arch with the mes-ethmoid. The inferior oblique of the right eye is attached to the ethmoid on the dorsal (morphologically left) side of this median arch and that of the left eye immediately behind that of the right. The large passage[1] between the ethmoid in front, the median arch at the right (morphologically dorsal), and the trabeculæ cranii at the left (ventral) shown in Figures *C′* and *D* has therefore in the growth of the cartilage been left to accommodate the oblique eye muscles, just as the olfactory foramina in the ethmoid were left because of the presence of the olfactory nerves.

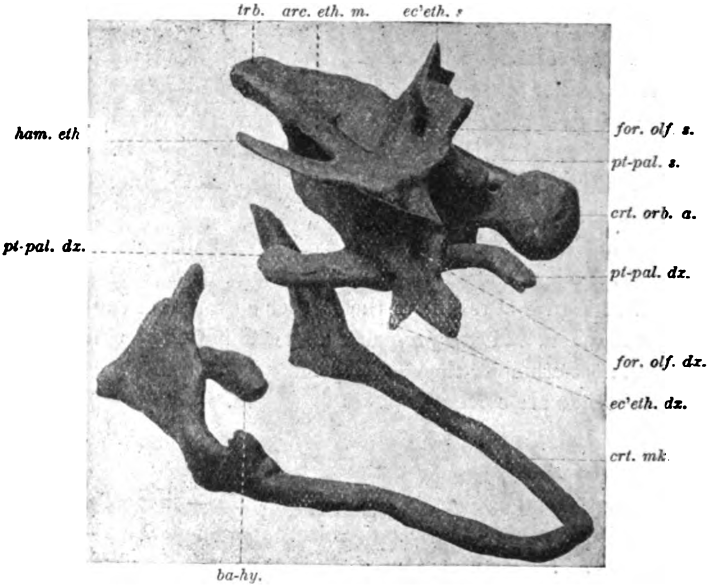

Fig. *D*.

Oblique view of the facial cartilages of *P. americanus*. Stage IV. Viewed from the same direction as in Figs. *A*. and *B*. × 70.
For meaning of lettering, see Abbreviations under Explanation of Plates.

The now ventrally projecting right ect-ethmoid partially hides in a lateral view the pterygo-palatine of its own side. The pterygo-palatine (Fig. *D*) ends abruptly at its posterior end, since the membrane bones which are to supersede it in supporting the upper jaw are already developed there.

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[1] This passage is seen in Figure *C′* directly above the pointed end of the probe inserted through the right olfactory foramen; it is indicated in Figure *D* by a triangular area at the right of the dotted line, *arc. eth. m.*

The left pterygo-palatine (*pt-pal. s.*) is visible in Figure *D* only in the region between the left ect-ethmoid and the cartilage sphere (*crt. orb, a.*) in front of the ethmoid. This terminal spherical mass of cartilage (*crt. orb. a.*) can be traced to its position in the adult skull. In a fish two inches long the ethmoid cartilage had pushed its way under this spherical cartilage, which had elongated in antero-posterior direction, but was still located between the nasal pits. I regard it, therefore, as the cartilage which forms in the adult the median anterior portion of the single orbit in which the left eye is to be found. The nasal bones lie on either side of it, and the rest of the orbit is made up of the right frontal, the left frontal and the left pre-frontal, or ect-ethmoid, bones.

By comparing the position of the olfactory openings in Figures, *B*, *C*, and *D*, it is plain that there has been a twisting of the ethmoid region from left to right, through an arc of 90 degrees. The line joining the centres of the ect-ethmoids in Figure *B* is horizontal, whereas in Figure *C* it makes with the horizon an angle of more than 30 degrees, and in Figure *D* is vertical. But with this twisting about the longitudinal axis the plane of the ethmoids has also revolved from a transverse position into one nearly coinciding with the sagittal plane, — possibly due to the pressure caused by the increase in the size of the eyes, — so that the axes of the olfactory foramina, which at first were parallel to the long axis of the fish, now pass from right to left. Accompanying these torsions, there has been a shifting in the relative positions of the olfactory foramina and surrounding cartilages till those of the right side are considerably in advance of those of the left. It is, however, the twist about the longitudinal axis which makes the migration of the eye seem rapid. This occupies in my experience not over three days, and according to Nishikawa ('97) it was completed in the fish which he observed in twenty-four hours.

The whole of the cartilaginous system of the facial region has been supported up to this time by two cartilage rods, the fused trabeculæ cranii (*trb.*, Figures *A–D;* Plate 1, Fig. 7; Plate 2, Fig. 10; Plate 3, Fig. 17) and the right supraorbital bar (*trb. su'orb. dx.*, Figures *A–D;* Plate 2, Fig. 10; Plate 4, Fig. 18).

The twisting is greatest in the optic region, the brain case showing little of it, and the anterior part of the ethmoid, as seen by the final position of the anterior ends of the pterygo-palatines, having turned not more than 45 degrees.

In the turbot, according to Traquair ('65, p. 276), the nasal region is nearly normal in position, the sagittal plane of the anterior part of the head nearly coinciding with that of the body.

f. *Comparison of Bothus with Pseudopleuronectes americanus.*

The nearest representative in American waters of the sinistral turbot is Bothus, the sand-dab, and I shall now compare briefly its turning with that of P. americanus. The sand-dab is much deeper than the flounder, but being thinner, though of the same length, it weighs about the same as that fish. Its translucency has gained for it the name of window-pane.

Traquair's statement that the turbot is less unsymmetrical than the plaice holds as truly here, the sand-dab being less distorted than the winter flounder. The mouth is straight and the length of the jaw on the ocular and eyeless sides is more nearly equal. The mouth is much larger and the gape greater than that of the winter flounder. The nasal pits are very nearly symmetrical, that of the right side being, however, a little the higher (Plate 3, Fig. 13). The transposed eye is not at all posterior to its mate, as is the case in P. americanus. The dorsal fin in this species reaches forward entirely past the right eye (Plate 3, Figs. 13, 16, *crt. pin. d.*). After the passage of the eye, the bases of the fin rays arise nearly over the right wing of the ethmoid.

The ethmoid is relatively a much more slender cartilage in Bothus than in P. americanus. The cross section of its anterior end (Plate 3, Fig. 13) has the shape of an inverted letter T, and its dorsal margin is turned not more than 20 degrees to the left from the sagittal plane. In the posterior region (Fig. 16) the ethmoid is turned about 45 degrees. The relation of the cartilage marked *trb. su'orb. s.* to the ethmoid mass in Figure 16 indicates the angle, though the median bar itself is farther forward. The wings of the ethmoid fuse to the median bar in a peculiar way. The right wing (*ec'eth. dx.* Fig. 13) points toward the rays of the dorsal fin which lie next it. It does not connect with the basal part of the ethmoid directly, but merely with the median upright part. The left wing has a process running anteriorly into the region of the lip at the level of the basal part of the ethmoid, with which this wing is fused. It then passes around the olfactory nerve of its own side, becoming much thinner as it does so, and unites with the upright bar. Thus the foramen for the left nerve (*I. s.*, Fig. 16) has a very thin outer wall, while for the right olfactory nerve (*I. dx.*, Fig. 16) there is no foramen. The olfactory nerves pass under the wings of the ethmoid to the capsules, which are located on the front faces of the wings.

Since the head of Bothus is less unsymmetrical than that of P. americanus, there is a corresponding difference in the conditions of the supraorbitals. The right supraorbital (Fig. 16, *trb. su'orb. dx.*) is crowded over until it comes to lie directly over the median bar of the ethmoid, which is continued backward into the interorbital septum. There it persists for a distance equal to nearly one-half the diamete rof the eye in all the specimens of Stage IV. (Bothus) which I have sectioned. It should be said that Bothus reaches this turned stage at a much earlier age than does P. americanus.

The left supraorbital is proportionately of larger diameter than the persisting supraorbital in P. americanus, and it also lies nearer the mesial arch, with which it is often connected. Such a connection sometimes occurs in the winter flounder, the condition of which has been previously described.

In the older specimens there is no separate supraorbital, but the upper end of the upright mesial cartilage bears a wedge-shaped enlargement on the side toward the left eye (Plate 3, Fig. 16, *trb. su'orb. s.*). When, in the more posterior sections, the mesial cartilage ends, this enlargement persists, and can be followed until it reaches the ear region, thus showing that it is the supraorbital cartilage. The cartilage forming the mesial arch is heavier and extends farther back between the eyes than in P. americanus. The result is as if some of the space between the hook and the trabecular cartilage in Stage IV. of P. americanus (*ham. eth.*, Fig. *D*) were filled out solid, and the whole plate were thickened.

In the transformation of the cartilaginous skull into the typical condition of the adult teleost, the skull bones, as is well known, may be formed (1) by ossification in the subcutaneous fibrous tissue (parostosis), or (2) by ossification between perichondrium and superficial cartilage cells, gradually replacing both by bone (ectostosis). There are no dermostoses, and, as in the case of the salmon (Parker,' 73), I saw no indications of endostosis. Of the bones directly involved in the turning, the frontals originate as parostoses and the pterygo-palatines and pre-frontals as ectostoses.

g. *Discussion of Pfeffer's Work.*

I have purposely omitted, up to this point, any comparisons with Pfeffer's work. He is the only author I have found who deals with the twisting in the larval Pleuronectidæ from other than the external point of view. Unfortunately, he does not give the name of the species on which his statements are based, nor are his papers illustrated.

In his earlier article ('86, p. 4) he describes the general conditions to be found in very young Pleuronectidae. The general topography is that of other young fish. The eye sockets — separated below by the sphenoid [trabeculæ cranii ?], above by the "Zwischenaugen-Decke" — communicate freely with each other in the intervening region. In the interorbital and ethmoid regions there is a vertical ridge-like dermal bone, having in cross-section the form of an elongated triangle, and supporting the dorsal fin, which, in Pfeffer's specimens, reaches to the ethmoid. This bone is still free from the cranium, and is the frontale principale of authors.

The bulbus olfactorius, which at first is lodged in the "Zwischenaugen-Decke," becomes crowded backward into the brain capsule. The "Interorbital-Decke" [supraorbital bar ?] is bent out toward the eye side and twisted somewhat on its long axis, so that its transverse axis, previously horizontal, now becomes oblique, slanting downward and outward toward the ocular side, while the chief part, which was vertical, is mostly resorbed by the migrating eye. As a consequence there now remains between the migrating eye and the surface of the head on the ocular side only the thin, glass-like, scarcely perceptible outer skin which previously covered the dermal bones. At the same time the dermal bone known as the frontale principale has grown fast to the interorbital roof-piece, and its course, at first straight from the median crest of the brain capsule to the ethmoid, now makes a great bend. Only its basal part, in the form of a broad band remains, while the vertical (and at first the larger) part has been resorbed. The upper part of the wing of the ethmoid on the ocular side has fused with the fronto-orbital, and the upper part of its outer margin is continuous with the now developing supraorbital cartilage or bone, while the wing of the eyeless side remains free on all sides, not forming any connection with the supraorbital of its own side.

This description of the relations of the wings of the ethmoid to the supraorbitals resembles the condition which I have found in Stage III *a* of P. americanus (Figure *B*, pp. 19, 20); but in P. americanus and in Bothus the dermal frontal is not yet present in the region through which the eye passes, and therefore cannot be resorbed. At Stage IV., *i. e.*, after the migration is practically completed, there is to be found in P. americanus under the surface of the skin behind the eye region a thin plate of bone, which I take to represent the left frontal. The supraorbital cartilage of the side from which the migrating eye comes lies in the region to which Pfeffer assigns the degenerating frontal in his species, and we have seen that this bar is resorbed. Perhaps in his species the dermal bone (frontal) is formed relatively earlier than in P. americanus.

Pfeffer's statement that the transposition of the eye is accompanied by a rotation on its own axis through an arc of 180 degrees is not quite correct for our species. The arc in P. americanus varies slightly in different individuals, but is approximately 120 degrees.

Neither will his theory of the formation of the "Knochenbrücke" fit the facts in Pseudopleuronectes. His argument (p. 8) is that when the frontal bone of the blind side changes its position, dermal bone is produced, not only over it in its new position, but also in the region of the integument beneath which the frontal was originally located, the latter dermostosis being known as the "Brücke." In our species at least, the frontal, when once formed, does not change its position. So its ontogenetic location does not explain the formation of the "Brücke."

In Pfeffer's more recent paper ('94) he states, as before, that very young symmetrical Pleuronectidæ have cartilaginous crania. The "Interorbitalbalken" [Interorbital-Decke ?] twists on its long axis, its dorsal edge toward the future ocular side. One eye moves downward while the other comes to lie upon the "Interorbitalbalken." If any sheathing bone is already formed on the "Interorbitalbalken," the elevated eye resorbs the part of the bone which is in its way. Then, on the side of the upper eye corresponding to the blind side of the adult fish there is formed a bony orbit, which fuses with the gradually developing dermal bones, so that the skull of such an individual leaves the false impression that the eye has traversed some of the bones of the skull.

The upper eye does not, according to Pfeffer, travel around to the other side of the skull, but ascends only a little, until on a level with the part of the skull between the eyes; however, from this time forward it looks in the direction of the ocular side. At the same time the thin piece of skin ("Körperhaut") now separating the cornea from the outer world, disappears.

In regard to the last point, I may say that in both species I find a layer of epidermis over the corneas of both eyes in the oldest fishes which I have sectioned, as indeed one would expect; so that Pfeffer's statement apparently would have been more accurate if he had said "Lederhaut" instead of "Körperhaut."

Unless the conditions in the species described by Pfeffer are totally different from those found in P. americanus and Bothus, Pfeffer has not distinguished between the cartilaginous supraorbital bar, which may be in direct connection with the cartilaginous wings of the ethmoid, and the dermal frontal bone, which fuses with ectostotic bone-tissue formed on the wings of the ethmoid.

h. *Résumé.*

The twisting which takes place in the ethmoid region of the skull of Pleuronectidae can best be explained by reference to the three mutually perpendicular axes of the head of the symmetrical young. There are two important torsions of about 90 degrees each. The most evident change (incidentally described by those who have discussed the migration of the eye) is that twisting of the ethmoids which can be represented by the revolution of the horizontal transverse axis until it approximately coincides with the original dorso-ventral axis.

The second change (limited to the upper part of the ethmoid mass) results in carrying the dorsal end of the dorso-ventral axis forward, so that it coincides with the longitudinal axis of the head. This change is probably due to growth along the anterior face of the ethmoids and resorption of the posterior dorsal margin, which is pressed upon by the eyes, or to a gradual displacement of the cartilage, due to the pressure referred to, without absorption.

In Pseudopleuronectes there is a further complication due to a slight retrocession of the parts on the eyeless side, amounting to about 30 degrees. This obliquity does not exist in Bothus.

The changes which have been described in the head of the flounder all take place in the cartilaginous skull, ossification occurring only after the shifting is complete. Therefore I cannot accept Pfeffer's view that a portion of the "frontale principale" lying in the path of the migrating eye is resorbed. The history of the two supraorbital cartilages links together to some extent the cartilaginous and bony conditions. The supraorbital cartilage bar next the migrating eye (the left in P. americanus, the right in Bothus) degenerates in its middle region, and the eye is carried through the gap thus made by the unequal growth of the facial cartilages of the two sides.

Later the ect-ethmoid of the "blind" side is formed as an ectostosis around the cartilage of that wing of the ethmoid and sends back a process along the line which the supraorbital cartilage had occupied. This meets and fuses with a forward process of the frontal of that side, thus forming the "Brücke," which becomes in the adult fish the most voluminous bony support of the nasal region.

The supraorbital of the other side keeps its connection with the ear-capsule much longer. Since the non-migrating eye moves downward to only a slight degree, the supraorbital has small space for movement to evade the pressure of the tissues in front of the migrating eye. So we find, in the latest stages in which this supraorbital appears at all, that the structures of the median plane have been crowded over upon the supraorbital and that this now appears as the cartilage "hook" (*ham. eth.*, Fig. *D*), which extends backward between the eyes and is at this time the chief tissue separating them.

In Bothus each frontal bone, when formed, sends forward a slender process between the eyes, but in P. americanus the process arises from the frontal of the ocular (right) side only.

V. The Optic Portion of the Central Nervous System.

1. General Condition in the Adult.

If the brain of the cod be taken for comparison, the axis of the cerebro-spinal part of the nervous system of P. americanus shows bendings that seem not to exist in the cod. There is in the spinal cord a bend which is convex upward (dorsad) and is apparently induced by the size of the digestive organs. In front of this, in the region of the medulla, occurs a bend which is convex ventrad (Plate 1, Fig. 6). Finally there is also a decided bend which is convex towards the eyeless side (Plate 2, Fig. 11). The muscles of the eyeless side being less developed, that side is more nearly flat than the ocular side, which is convex.

Figure 8 (Plate 2) is a dorsal view of the brain of a fish (P. americanus) three inches long. The curves mentioned are not yet emphasized. An evident sign of asymmetry is seen in the inequality in the size of the olfactory lobes, that of the right side being much the larger. This lobe may, in the adult, have six times the volume of that of the left side (compare Fig. 11). The relative sizes of the lobes of the cerebrum is different in different individuals. In the specimens shown in Figures 8 and 9 (Plate 2) and in Figure *F* (p. 36) the left lobe is the larger; but in a number of adult fishes the right lobe was the larger.

The optic lobe of the left side is usually cut first in cross-sections, when one begins the cutting at the anterior end of the animal, as is plain from the relative positions of the two in this specimen (Fig. 8). The course of the optic nerve to the transposed (left) eye is shown by dotted lines (*II. s.*) in the figure. Its slack condition allows the eyes to be thrust upward when the fish is buried in the mud or sand. One or two movements of the fins will cover a fish with loose sand; except for the projecting eyes, the animal is then entirely concealed. This protrusion of the eyes is done by means of the so-called orbital heart. This organ, mentioned by Agassiz in his description of the developing flounder, is described as the recessus orbitalis by Holt ('94). It is shown in cross section at *rec. orb.* in Figure 18 (Plate 4).

A side view of the same brain as that shown in Figure 8 (Plate 2) is seen in Figure 9, which makes clearer the position of the brain with reference to the eyes; but in the dissection the left eye has been raised somewhat from its normal position in order to show the eye muscles and the location of the optic nerves, which are purposely shaded somewhat darker than the surrounding muscles.

In all the flatfishes which I have examined, the optic nerve from the transposed eye is dorsal (anterior) in the chiasma. In P. americanus the right optic tract and the left optic nerve are anterior (dorsal) to the corresponding parts of the opposite sides (Fig. 12), whereas in Bothus the left tract and the right nerve are anterior (dorsal).

Figure 11 is drawn from a dissection of the adult fish. The oculomotor nerve (*III.*) supplying the transposed eye passes toward the eyeless side before it divides into the four customary branches. The fourth cranial nerve (*IV.*) is still more noticeably changed in its direction. In the cod this nerve lies near the median plane, at a distance from and above the eyeball; but in the flounder the fourth nerve of the migrating eye lies in contact with the eyeball and rests on the dorsal rectus muscle. The optic nerve (Figs. 8, 11) also shows before reaching the eyeball a bending in the same direction as that which the eye-muscle nerves exhibit. These alterations in the directions of the nerves in the adult indicate the nature and the place of the transposition which we have followed in the larvæ, and show that nerves retain throughout life, as far as possible, their phylogenetically normal position. I was unable to find from my dissections that the flounder, P. americanus, has a cutaneous branch of the fifth nerve. If it has, the nerve must be small. The fifth has a mandibular, a maxillary and a superior ophthalmic branch. The large ophthalmicus profundus of the cod is represented in the flounder by a few twigs only (*V. opt. p'fnd.*, Fig. 11). The left superior ophthalmic of the flatfish (*V. opt. su.*), after emerging from the skull with the rest of the fifth nerve, as in the cod, runs from left to right (Fig. 11) *through* the passage formed by the "Brücke," which results from the fusion of the posterior angle of the pre-frontal and the corresponding anterior angle of the left frontal. It then takes the regular median path between the eyes to its distribution on the snout. The bone is formed around the nerve in its new position after the migration of the eye.

The seventh nerve in both the cod and the flounder emerges from the skull with the fifth. The ninth in the cod lies between the two chief roots of the tenth, with which it passes out. In the flounder the ninth nerve lies in front of the tenth and passes through the ear capsule to its distribution on the hyoid and first gill arch.

2. THE OPTIC NERVES.

In the cross-section of a fish in Stage I. (Plate 3, Fig. 17), one section, 10μ thick, contained the whole length of both optic nerves from the blind spot to the chiasma. The blind spot is very near the outer ventral edge of the retina and in about the middle of the eye antero-posteriorly. Therefore the chiasma is in the transverse plane which passes through the middle of the eyes. There is, as yet, scarcely any want of symmetry, the left eye being only slightly higher than the right.

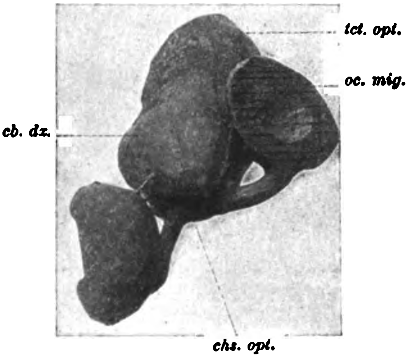

FIG. *E*.

A precisely front view of the fore part of the brain, the optic nerves and a portion of each of the optic cups, modelled in wax (Born's method) from a specimen in Stage III. × 50.
For explanation of lettering, see Abbreviations under Explanation of Plates.

I have no corresponding illustration of the condition of the optic nerves in Stage II., but a model of the anterior part of the brain and the optic nerves of a specimen in Stage III. *a* is shown in Figure *E* (the anterior portion of the left optic cup has been omitted in the model; the cut surface being indicated by horizontal lines). The left eye is higher than the forebrain; its ventral edge is at the same level as the dorsal side of the right eye, and the transverse plane tangent to its posterior surface would cut the right eye about midway between its anterior and posterior faces. The right eye may have moved slightly ventrad from the position which it occupied in Stage I. The slackness of the nerves is shown by the curve that they take as they pass forward and outward. The whole of the midbrain and most of the forebrain have lost their earlier position between the eyes, owing to the growth in length of the facial cartilages. Figure 9 (Plate 2), a side view of the brain of a fish three inches long, shows this antero-posterior separation between

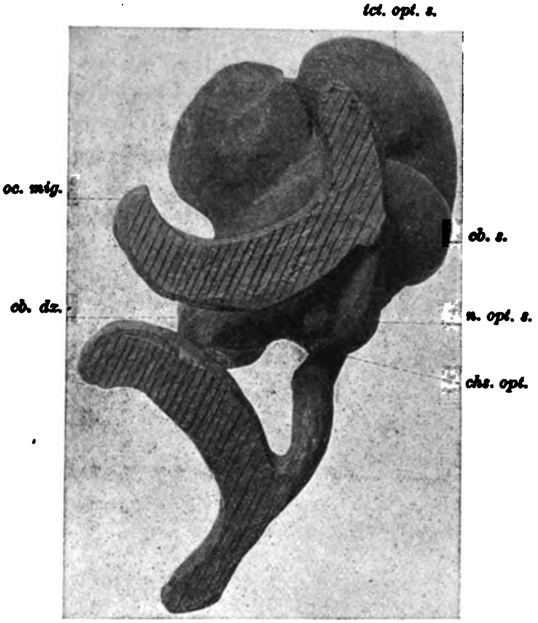

Fig. *F*.

Front view of the fore part of the brain, the optic nerves and portions of the optic cups in Stage IV. From a model (Born's method). × 50. Compare Fig. *E*.

For meaning of lettering, see Abbreviations under Explanation of Plates.

brain and eyes farther advanced, and Figure 11 (from an adult) shows it completed.

In the essentially adult condition of Stage IV., as shown in a front view of the modelled brain and optic nerves (Figure *F*), the left eye has passed so far to the right side that, taking into consideration the high degree of mobility of the eye its field of vision almost coincides with that of the right eye. The optic nerves curve still more in their passage from chiasma to eye, and the distance is proportionately greater. The right cerebral lobe (*cb. dx.*) is seen in the figure between the eyes, and the left cerebral lobe (*cb. s.*) is seen on the right, behind the left eye, and below the tectum. The left olfactory lobe is covered by the left eye, but the right olfactory lobe — modelled as a continuation forward of the right cerebral lobe — is seen between the two eyes. The left optic lobe (*tct. opt. s.*) in both these instances (Figures *E* and *F*) extends farther anteriorly than the right. This is seen in the dorsal view of the brain (Fig. 8). This figure also shows why in making cross-sections the left lobe of the cerebrum is cut before its olfactory lobe in case one begins at the anterior end.

The optic nerve — round in cross-section in the larvæ — becomes thrown into folds in the adult (Plate 5, Fig. 24). This condition is also figured by Studnicka ('97) for one of the Pleuronectidæ. The cross-section may show as many as six or seven folds closely pressed together. Small neuroglia nuclei are scattered throughout the length of the nerve.

3. THE CHIASMA AND TRACTS WITH RELATED GANGLIA.

The optic crossing is complete as in all teleosts. There is no interlacing of fibres, as can be seen in Figure 19 (Plate 4), which is from a fish in Stage IV. This is an approximately transverse section, which, however, cut the left side of the fish somewhat farther caudad than it did the right side. The plane of the section also inclines a little backward and upward, so that it coincides with the plane of the anterior part of the left optic tract, which slants in Figure 19 backward and upward on its way to the tectum. The right tract is cut crosswise, nearly at right angles to its course. (This is by mistake lettered *n. opt. s.* in Figure 19. Of course, as it is posterior to the chiasma, it should have been labeled *trt. opt. dx.* For the second section anterior to this the label *n. opt. s.* would be correct.) The median, dorsal portion of the tract (*trt. opt. d.*) passes upward through the nidulus corticalis (to be described later) on its way to the median portion of the tectum. The external, ventral portion (*trt. opt. v.*) passes outward and around to its distribution on the posterior, lateral, and ventral tectal surfaces.

The geniculate body (Figs. 20, 21, *cp. gnic.*) lies in the angle between the two portions of the Y-shaped tract, but almost entirely in front of their plane. There is some indication of a division of the corpus geniculatum into anterior and posterior parts.

In both Weigert and Congo-red preparations it could be seen that a few optic fibres entered the geniculate bodies (Plate **4**, Fig. 21). C. L. Herrick (**'92**, p. 430) found no ending of optic fibres before reaching the tectum. This ending has been demonstrated, however, by Mayser (**'81**) in Cyprinoids, by Auerbach (**'88**) in the trout, by Haller (**'98**) in Salmo, and by Krause (**'98**), who used Marchi's method for degenerate nerves, in Cyprinus auratus. Edinger (**'96**, p. 126), makes the following statement for vertebrates. "Im Geniculatum [laterale] endet ein Theil des Sehnerven mit mächtiger Aufsplitterung, und mitten in diese Faserung tauchen die Dendriten langgestreckter Doppelpyramiden. Das mediale Ende dieser Pyramidenzellen splittert auf in einem Zuge, der wahrscheinlich auch dem optischen System angehört."

I have no Golgi preparations which show optic fibres actually fibrillating in these bodies. There was, however, in the geniculate bodies but one type of cell impregnated with the chrome-silver. This was a small unipolar cell (Plate **5**, Fig. 22) with a short process ending in very thick short fibrillations directed towards the end of the geniculate body into which the optic fibres enter. In a single exceptional instance, a cell, otherwise like the ones described, had another short but unbranched process extending in the opposite direction (see diagram of tectum, Plate **5**, Fig. 22, *cp. gnic.*).

Fusari (**'87**), after a study of Carassius, Macropodus, Anguilla, and Lopodogaster, stated that in his opinion fibres from the tractus pass through the corpus geniculatum and unite again with the tract to fibrillate in the tectum. No preparations of P. americanus indicated such a possibility.

No other bundle of fibres could be found to leave the tract before it reached the tectum itself. Mayser (**'81**) describes a small bundle passing into the thalamus at about the point of origin of the paraphysis. Auerbach (**'88**), Mirto (**'96**), and Haller (**'98**) also indicate a thalamus bundle, and Haller describes a small bundle running to the fore-brain. In my opinion Mayser, Auerbach, Mirto, and Haller have mistaken a portion of the ventral division of the tract, which bends outward sharply in its course to the ventral posterior part of the optic lobes, for a thalamus bundle. In parasagittal sections the cut ends of this portion of the tract appear to be pointing into the thalamus. But no one of these authors has described fibrillations or cell endings for this thalamus bundle, and the absence of degeneration in Krause's experiment would indicate that Mayser's thalamus root was non-optic.

A frontal section (Plate 4, Fig. 20) shows the relation of the thalamus ganglia to the tectum. The geniculate bodies lie anterior to the lobes of the tectum, and between them are the ganglia habenulæ (*gn. hab.*), which bound the third ventricle, and are separated from each other by the pineal-gland region. A few sections dorsal to the one shown in this figure the habenular commissure appears.

As Haller ('98) has found in the case of Salmo, the habenulæ are symmetrical, in the young fish at least. Because of the want of symmetry in older brains it is impossible to obtain single sections in which one is certain that the habenulæ are cut in like planes. In a cross section which passes through both ganglia the left ganglion has a greater dorso-ventral diameter than has the right, while the right ganglion measures more from side to side than the left.

In Figure 20 the fibres of the two parts of the optic tracts are shown in cross-section behind the edges of the geniculate bodies. Also behind the geniculate bodies lie large cells which belong to the nidulus corticalis of Fritsch, the "Dachkern" of Edinger and others.

Since fibres from this nidulus enter the tectum, I will describe its location more particularly in the two Pleuronectidæ studied. There are two symmetrically placed groups of very large ganglionic cells lying at the front part of the tectum; they extend anteriorly from the angle of the optic ventricles, where the lobe of the tectum and the axial portion of the midbrain meet, to the outer surface of the brain above and outside the geniculate bodies. There is no difficulty in identifying the cells of the nidulus (*nid. ctx.*, Plate 5, Fig. 23), as they are pear-shaped and many times larger than those of the gray layer of the tectum, into which the posterior portion of the nidulus extends.

The nucleus lies in the blunt end of the pear-shaped cell, at the end opposite the coarse cell process. Since these processes gather into bundles in the middle layers of the tectum, the nucleated ends of the cells are directed towards the surface when the cells are more superficial, but toward the optic ventricles if they are deep (compare Fig. 22).

There is a similar nidulus, consisting of a few (20–30) even larger cells, which lies ventral and exterior to the nidulus corticalis; it lies posterior to, but in contact with the optic tract. This possibly is the nidulus anterior of Edinger, though I have traced no fibres from it. A few cells of this nidulus are shown between the two portions of the tract in Figure 19 (Plate 4).

In one instance I found a cell of the nidulus corticalis which sent a fine process, probably a neurite, ventrad with the other fibres of the optic tract (Plate 5, Fig. 22). This could be followed nearly to the chiasma, but whether it continued to the eye or bent backwards into one of the post-optic commissures, I cannot say.

I can confirm C. L. Herrick ('91–'92) in his statement that the commissura horizontalis (*coms. hz.*, Plate 5, Fig. 22) arises from the nidulus corticalis. The fibres forming this bundle were fine and took the same quality of Golgi impregnation as the single fibre just described from one of the cells of the same nidulus which passed downward through the tractus opticus. The fibres composing this bundle can be followed in two or three parasagittal sections to the nucleus rotundum of the same side; they pass through this nucleus, and then turn forward and cross to the opposite side behind the chiasma as the horizontal commissure.

4. THE TECTUM OPTICUM.

Since the tectum is that portion of the brain in which the optic tracts terminate, it should be the place in which the transition from sensory to association or motor neurons takes place.

There are certain points of interest which can be shown from a surface view. At the anterior ends of the tectal lobes, in P. americanus, but not in Bothus, there is an exterior furrow or sulcus (*sul. tct. opt.*, Plate 2, Fig. 11), much like one that is found in the cerebrum of simple type — in that of a turtle, for example. This gradually disappears toward the posterior region of the tectum. Cross-sections in the anterior region show that this sulcus is due to a lateral horizontal depression in each optic lobe, which divides it into almost equal dorsal and ventral parts. The ventral portion of the tractus supplies the ventral half of the lobe and the dorsal portion the dorsal half. The geniculate bodies lie in the region of greatest constriction of the tectum.

For convenience, I divide the tectum into seven layers, indicated by the numerals 1–7 (Plate 5, Figs. 22, 23), in addition to the membranes of the brain, which are the vascular connective-tissue layer (the arachnoid, *mb. ach.*) and, beneath this, a very thin membrane, the pia, to which the endings of the ependymal cells reach, and along which is found here and there a nucleus.

Passing from without inward, the tectal layers are as follows:

(1) A thin outer layer, composed principally of nerve fibrillations with a few nerve cells. In this layer the ependymal fibrillations end. A corresponding layer is recognized by writers on the finer anatomy of the tectum in the bony fishes, from Stieda ('67) onwards, except by Fusari ('87, '96) and Van Gehuchten ('95). Fusari ('87) described a layer of vascular connective tissue beneath the pia, and later ('96) his first layer of the tectum was made to embrace this vascular layer and the optic-fibre layer.

(2) The layer of the medullated optic fibres. This is the continuation of the optic tract and is recognized as a separate layer by all writers on the tectum.

(3) A layer of optic fibrillations. This is not made a distinct layer by Stieda ('67), but Mayser ('81) and nearly all writers since his time have emphasized its presence.

(4) A spindle-cell layer.

(5) The fillet layer, composed of longitudinal fibres and cross commissural fibres. Stieda considered the fibres, which here run in two directions, as two layers. C. L. Herrick ('91-92) describes a layer of commissural fibres *beneath the fillet* connecting the two optic lobes.

(6) The "gray" layer.

(7) The reticulate and ependymal layer. Some authors consider that this is composed of two distinct layers. The reticulate portion is not described at all by Neumayer ('95), Van Gehuchten ('95) nor Edinger ('96).

Mirto ('96) based his division of the tectum into layers on the *shapes* of the cells which he was able to demonstrate by the Golgi method. Following Cajal's work on the tectum of birds, he describes fourteen layers.

The degeneration methods did not yield much of importance in my hands, although the flounder, owing to its habit of protruding the eyes, is a favorable fish on which to operate. The animals, even the very small metamorphosed fishes, stand the shock of the removal of the eye well and bleed very little from the operation. The specimens tried by the Marchi method were very brittle, and demonstrated but one point clearly, that the sixth (nerve-cell) layer was reduced. Fusari ('96), who used the Weigert-Pal staining method on a Cyprinoid, concluded that all the tractus fibres degenerated when the eye was removed. Krause ('98), after the Marchi treatment of fish from which the eyes had been removed, found that about one-tenth of the tract — mostly distributed in the dorsal root, which spreads on the roof of the tectum — did not degenerate. In a very old one-eyed fish both the geniculate ganglion and the torus longitudinalis were, he found, much atrophied and the fillet was reduced. The spindle-cell layer contained fewer cells than were found in fishes more recently operated on.

Turning next to the finer anatomy of the tectum a diagrammatic representation of a parasagittal section is shown in Figure 22 (Plate 5). This exhibits the types of cells found in the tectum by the aid of the silver method.

In layer 1 few cells were impregnated. Of these the more common type (Fig. 22, α) was oval and bipolar, its two processes running parallel to the fibres of layer 2. In some instances, however, the cell had a third and even a fourth process. Similar cells have been described by Fusari, except that the cell bodies described by him were spherical. Neumayer ('95) has shown elongated bipolar cells with processes parallel to layer 2, and also rounded cells whose neurites fibrillated in the layer of optic fibres. Mirto ('96) indicated cells in corresponding positions, but with triangular bodies. I, also, have found a few pear-shaped cells (Fig. 22, β) in this layer. These lay near the surface and sent off their processes from their deeper, smaller ends. Some of these processes passed through the optic layer (2) into layer 3, while others turned at right angles and ran in layer 1 parallel to the surface.

Layer 2 is composed of the medullated fibres which enter the tectum as the optic tract. At the beginning of the tectal region the fibres of the tract, after having passed beneath the geniculate body, bend toward the surface of the brain to form this second layer. Some of the cells of the nidulus corticalis (*nid. ctx.*) lie in this layer, since the nidulus extends from ventricle to surface. The bulk of the dorsal bundle of fibres from the tractus passes too near the sagittal plane to touch the nidulus corticalis, and the ventral division does not reach as far dorsally as the nidulus. So there is little disturbance in the course of the fibres of the tractus in passing these very large cells. The diminution in the thickness of the optic-fibre layer in passing from before backwards, which is due to the fibres continually spreading out over more of the surface of the optic lobe, and to the termination of many of them in anterior regions, is shown in Figure 25 (Plate 5).

Here and there other cells, besides those of the nidulus corticalis, which lie at the anterior end of the tectum, are seen in the optic layer; these have fibres, some of which extend inward, others outward. The cell-body of one of these (Fig. 22, γ) was pear-shaped, the smaller end being directed outward. From this smaller end processes ran both anteriad and posteriad, the most of them parallel to the surface; one, however, took an oblique direction, running forward and inward, and reached layer 3. Neumayer represents in this optic layer spindle-shaped cells, the upper ends of which fibrillate in layer 1, and the lower in layer 3.

The third layer contains cells of many shapes. (*a*) Short spindle-shaped cells (Fig. 22, δ) with one process directed outward and fibrillating in layer 1, and one or more processes directed inward. Cells like these are described by Fusari, Neumayer, and Mirto, and the last two authors say that the neurites are directed inward and reach the fillet layer. Fusari also describes a type of cell which is spindle-shaped with processes extending downwards and fibrillating just above the fillet layer. A neurite of one of these cells is figured running through the corona radiata of Gottsche[1] into the torus semi-circularis. (*b*) Pyriform cells (Fig. 22, ε) with all the processes directed inward and the ends of the fibrillations reaching into layer 4. (*c*) Rounded cells (Fig. 22, ζ) with rather long sparsely branched processes, the outward process having been followed in one case into the optic-fibre layer. (*d*) Cells (Fig. 22, η) the reverse of those denominated ε in this layer, with fibrillations having the opposite direction and reaching to, or even through, the optic layer into layer 1. (*e*) Lying near the boundary between this (3) and the next deeper (4) layer were found a few cells (Fig. 22, θ) flattened in a direction perpendicular to the surface of the optic lobes. Each of these possessed a process running from either end parallel to the surface of the tectum and sometimes a third one passing out towards the surface. At or near this transitional region between layers 3 and 4 the fibres from most cells send off short branches parallel to the surface.

I have separated layers 2 and 3 because in the anterior portion of the tectum some fibres from the optic tract take a direct course into layer 3 without first bending outward into layer 2. In the posterior portion of the tectum, however, it is not possible to distinguish these two layers.

Bundles of large processes from the nidulus corticalis (*nid. ctx.*) enter the anterior portions of these two layers and form a prominent fibrillation, traceable for some distance backward. These coarse, wavy processes are much larger than the fine fibres, which I have shown (p. 40) to be the neurites which make up the horizontal commissure, and there may be two or three of them from one cell. These coarse processes can be followed backward for some little distance along distinct paths in layers 3 and 4, and the general appearance of the fibrillations farther back indicates that these processes, branching continually, pass backward through the tectum much farther than continuity can be directly traced. A dendrite may branch and follow the fibrillar paths in each of the two layers.

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[1] This is the "Stabkranz," the descending fillet fibres.

A large system of fibres also enters the same general region of the tectum from the axial part of the mid-brain; some of these cross from the opposite side of the brain in the lower part of the posterior commissure. These fibres may constitute the most anterior portion of the commissura mesencephali (Herrick's sylvian commissure) or, as I think more likely, they may come from the motor regions, possibly Haller's anterior connective. I have not succeeded in tracing these fibres to any cells.

In layer 4 appear the cells which are most characteristic of the tectum (Fig. 22, ι). They were impregnated in most of the Golgi preparations. They are spindle-shaped, being much elongated in a radial direction, and have fibrillations which extend outward as far as layer 2. Sometimes there is an impregnated process which goes from the deeper end of the cell into layer 5, and sometimes there is not. Neumayer and Mirto each state that the neurites of these spindle cells are traceable to the fillet layer and the fibrillations to the optic layer. Mirto describes cells with the same processes but with much more slender bodies. The spindle-shaped bodies are shown by my hematoxylin preparations to be very abundant indeed in this layer, only a few taking the Golgi impregnation in a single specimen. In this layer (4) there were also found sparingly cells (Fig. 22, κ) with rounded bodies and processes which fibrillate inwards and extend into the fillet layer (5). A very few pyriform cells lie near the deep surface of this layer (4) and send their processes outward (Fig. 22, λ). Fusari shows irregular, large-bodied cells with many processes and neurites, when such are present, extending into layer 5. A bifurcate cell is figured by Mirto with its telodendrites in layer 3. My flounder impregnations produced neither of these types.

I have spoken of layer 5 as the fillet layer because it is composed chiefly of fibres which pass backward and medianward, forming the so-called corona radiata of Gottsche, the lemniscus or fillet system.

This layer is composed of cross and longitudinal fibres which, seen in tangential section, form a meshwork over the whole of the dorsal part of the tectum. In front of the optic ventricles bundles of fibres (Plate 5, Fig. 22, *lmn.*) can be followed from the axial part of the mid brain through the region of the nidulus corticalis into the longitudinal fibre layer. Most of the cross-lying fibre bundles, which form the commissura mesencephali, lie below the longitudinal layer. Some of these cross bundles seem to turn longitudinally after crossing the mid-line. It may be that the uncrossed fibres of the fillet are a continuation of these. The longitudinal fibres, at any rate, pass back in bundles to the region of the anterior peduncles of the cerebellum. In any section which cuts through the whole thickness of the tectum, whether cross or parasagittal, some bundles will be shown (Plate 5, Fig. 25, *lmn.*). As the tectum is dome-shaped, the more nearly median parasagittal sections will cut the fibre bundles at the anterior and posterior ends of the tectum, whereas the more lateral sections will show the fibres of the middle of the tectum cut longitudinally. There is a rather distinct portion of the fillet which arises from the anterior ventral part of the tectum and, slanting upwards and inwards, passes through the nidulus-corticalis region back towards the cerebellum, beneath and behind the median boundary of the optic ventricles. The fillet fibres may be roughly likened to the slightly curved fingers of an open hand, palm inward, wrist beneath the cerebellum, grasping the most of the gray layer of the tectum. The gray of the posterior portion of the tectum seems, however, to be outside the region surrounded by the fillet-fibre bundles.

The fibres of the commissura mesencephali cross just above the gray layer in the anterior part of the tectum in the region of the torus longitudinalis. According to Herrick they form a continuation of the series found in the posterior commissure.

Besides these fibres, there are in layer 5 a number of different forms of cells: (*a*) Cells with rounded bodies (Plate 5, Fig. 22, μ) of the same size as those (Fig. 22, ρ) in the next deeper layer (6) — the gray layer — and with processes which may fibrillate into any one or all of the more superficial layers (1–4) of the tectum. (*b*) Spindle-shaped cells (Fig. 22, ν) like those (ι) characteristic of layer 4. When an axonic process can be followed from the deep end of such a cell, it finds its way into the fillet layer, but whether into the cross or longitudinal system I cannot determine. (*c*) Long triangular cells (Fig. 22, o) with a single process extending toward the periphery, and from each of the corners of the deep end a process running parallel to the fillet layer. (*d*) Rounded cells (Fig. 22, π) with fibres which turn immediately into the fillet layer and with very short dendritic processes.

The next layer (6) is the gray molecular or granular layer. This is the most noticeable portion of the tectum, especially in young animals. The nuclei are closely crowded together, with a definite arrangement due to the radially directed processes of the ependymal cells, which pass through all the layers from the ventricle to the pia. Only one type of cell body (Fig. 22, ρ) is evident, that being the small and rounded form; in Golgi preparations, it is slightly pear-shaped, and resembles much the ependymal cell. But since the cells of this layer have processes of a number of types, they cannot all be, as Fusari ('96) maintained, ependymal cells. They may fibrillate in any or all of the layers outside the sixth. In Golgi preparations a very few spindle cells, like those in layers 4 and 5, appear. Some of the peripheral cells (Fig. 22, σ) of this layer, as well as the very deep ones, may send to the surface a process which ends in branching fibrillations beneath the pia. The fibres from other cells were found to break up in layers 3, 4, and 5. These fibres are often impregnated when none of their processes take the silver, or vice versa. The cells next to adjacent layers, whether the deeper or those nearer the periphery, are more likely to become impregnated than those in the middle of the layer.

The innermost layer (7), less dense than any of the preceding, is composed of the bodies of the ependymal cells and the basal portions of their processes. A reticulate portion of this layer (next to layer 6) is not apparent in young specimens, and so I have not recognized it as a separate layer, but have included in layer 7 all that lies between the gray layer (6) and the ventricle.

In the adult brain there are scattered through this loose layer a few large-bodied very irregular cells (Fig. 22, τ), each having a multitude of long beaded processes. I was unable to discover any neurite connected with these cells.

In order to simplify the diagram (Fig. 22), I have omitted in all cases the free fibrillations. In most impregnations where there are any at all, there are so many that only a few can be traced to any definite medullated layer. Layer 3, however, certainly contains, among other fibrillations, free branches from the optic layer (2). In layers 3 and 4 free fibrillations of fibres from cells in layer 5 are doubtful, because any one of the many cells in the granular layer (6) may have its fibre impregnated though itself remaining clear.

Between the fillet layer (5) and the optic layer (2) there are two especially dense fibrillar regions corresponding in general to the two bundles of dividing processes which arise from the cells of the nidulus corticalis.

For the purpose of comparing the impregnation of the tectal region in these Pleuronectidæ with that of the same region in a symmetrical fish, in order to ascertain whether there are any noticeable histological differences, I have applied the Golgi method to the brain of Fundulus heteroclitus, the mud minnow. These were found to take the stain very much more easily than do flounders; but there was also more of the silver precipitate carried inward from the surface. I conclude, therefore, that the tissue in Fundulus must be more open. Except as to the size of certain cells and the relative thickness of some fibre bundles, the two brains correspond closely. The cells of the nidulus corticalis in the minnow are much smaller proportionately, though their tectal processes can be followed in layers 3 and 4 as far as in the Pleuronectidæ. The spindle-shaped cell found most abundantly in layer 4 was again in the minnow the most noticeable cell impregnated, and was found most often. A triangular cell in layer 5, very similar to the cell *o* found in the corresponding layer of the flatfish, had its outward process extended to layer 1, where it fibrillated like an ependymal cell.

Most of the cells of layers 3, 4, and 5 in Fundulus had neurites traceable into layer 5, the fillet layer.

VI. Theoretical Considerations.

The conditions in the tectum are the same as those found in the optic lobes of typical Teleostei. The division of the tectum into layers is of importance as a means of more precise description. There must be a place where the fibres of the optic tract, which come in as layer 2, end; that region is layer 3. There must be an association system connecting with the posterior motor regions, and the fibres of this system are either a part or the whole of layer 5. If only a part, then the purpose of the commissura mesencephali is to put the two optic lobes in communication with each other. The cells in layers 3, 4, and 6, especially the spindle cells in layers 3 and 4, probably serve to receive and transmit optic stimuli.

The nidulus corticalis, developing early, as it does, is probably one of the most effective association centres of the brain. Lying at the entrance to the tectum, with a strong bundle of neurites running through the two niduli rotundi in the ventral part of the brain, and with its numerous large dendrites passing into layers 3 and 4 of the tectum, it should be able to connect the optic sensory region with the motor areas quickly, and thus account for the extreme rapidity of movement of these larvæ.

The "why" of the peculiar metamorphosis of the Pleuronectidæ is an unsolved problem. The presence or absence of a swim bladder can have nothing to do with the change of habit of the young flatfish, for P. americanus must lose its air-bladder before metamorphosis begins, since sections showed no evidence of it, whereas in Bothus the air-sac can often be seen by the naked eye up to the time when the fish assumes the adult coloration, and long after it has assumed the adult form.

Cunningham (**'92-97**) has suggested that the weight of the fish acting upon the lower eye after the turning would press it towards the upper side out of the way. But in all probability the planktonic larva rests on the sea bottom little if at all before metamorphosing. Those taken by me into the laboratory showed in resting no preference for either side until the eye was near the mid-line.

That the change in all species is repeated during the development of each individual fish, has been used to support the proposition that the flatfishes as a family are a comparatively recent product. They are, on the other hand, comparatively ancient. According to Zittel (**'87-90**, pp. 315-316) flatfishes of species referable to genera living at present, Rhombus and Solea, are found in the Eocene deposits. These two genera are notable in that Rhombus is the least and Solea the most unsymmetrical of the Pleuronectidæ.

The degree of asymmetry can be correlated with the habit of the animal. Those fishes, such as the sole and the shore-dwelling flounders, which keep to the bottom, are the most twisted representatives of the family, while the more freely swimming forms, like the sand-dab, summer flounder and halibut, are more nearly symmetrical. Asymmetry must be of more advantage to those fishes which grub in the mud for their food than to those which capture other fishes; of the latter, those that move with the greatest freedom are the most symmetrical.

This deviation from the bilateral condition must have come about either as a "sport," or by gradual modification of the adults. If by the latter method, — the change proving to be advantageous, — selection favored its appearing earlier and earlier in ontogeny, until it occurred in the stages of planktonic life. Metamorphosis at an age younger than this would be a distinct disadvantage, because of the lack of the customary planktonic food at the sea-bottom. At present some forms of selection are probably continually at work fixing the limit of the period of metamorphosis by the removal of those individuals which attempt the transformation at unsuitable epochs, — for instance, at the time of hatching. That there are such individuals is shown by Fullarton (**'91**), who figures a fish just hatched "anticipating the twisting and subsequent unequal development exhibited by the head of Pleuronectids." Those larvæ which remain pelagic until better able to compete at the sea bottom become the adults which fix the time of metamorphosis on their progeny.

VII. Summary.

1. The young of Limanda ferruginea are (probably) in the larval stage at the same time as those of Pseudopleuronectes americanus.
2. The recently hatched fish, both P. americanus and Bothus, are symmetrical, except for the relative positions of the two optic nerves.
3. The first observed occurrence in preparation for metamorphosis in P. americanus is the rapid resorption of the part of the supraorbital cartilage bar which lies in the path of the eye. This is probably due to pressure from the migrating eye.
4. Correlated with this is an increase in the distance between the eyes and the brain, caused by the growth of the facial cartilages.
5. The migrating eye moves through an arc of about 120 degrees.
6. The greater part of this rotation (three-fourths of it in P. americanus) is a rapid process, taking not more than three days.
7. The anterior ethmoidal region is not so strongly influenced by this twisting as the ocular region.
8. The location of the olfactory nerves shows that the morphological mid-line follows the inter-orbital septum.
9. The cartilage mass lying in the front part of the orbit of the adult eye is a separate anterior structure in the larva.
10. With unimportant differences, the process of metamorphosis in the sinistral fish is parallel to that in the dextral fish.
11. The original location of the eye is indicated in the adult by the direction first taken, as they leave the brain, by those cranial nerves having to do with the transposed eye.
12. The only well-marked asymmetry in the adult brain is due to the much larger size of the olfactory nerve and lobe of the ocular side.
13. There is a perfect chiasma.
14. The optic nerve of the migrating eye is always anterior to that of the other eye.
15. The optic tract is divided into dorsal and ventral portions.
16. There are fibres from the tract which enter the geniculate body. No other bundles of fibres leave the tract before it reaches the tectum.
17. The ganglia habenulæ are symmetrical, at least in the larva before metamorphosis.

18. There is a notable sulcus on the lateral side of the adult optic lobe, which increases the surface area of the tectum.

19. The nidulus corticalis is the origin of the horizontal commissure and of a large bundle of nerve fibres which pass into layers 3 and 4 of the tectum.

20. The most important receiving cells for the fillet layer are probably the large spindle cells in layer 4.

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EXPLANATION OF PLATES.

Figures 13, 14, and 16 are of *Bothus maculatus*. All others are of *Pseudopleuronectes americanus*. All except Figure 11 were outlined with the camera lucida.

ABBREVIATIONS.

a.	Anterior.
an.	Anus.
arc. eth. m.	Mesial cartilage arch of the ethmoid.
ba-hy.	Basi-hyal.
can. smi'crc.	Semicircular canals.
cb. (*dx.*, *s.*)	Cerebrum (right lobe, left lobe).
cbl.	Cerebellum.
chs. opt.	Optic chiasma.
cl. crt.	Degenerating cartilage cells.
coms hz.	Commissura horizontalis.
cp. gnic.	Geniculate body.
crt. mk. (*dx.*, *s.*)	Meckel's cartilage (right, left).
crt. orb. a.	Antorbital cartilage.
crt. pin. d.	Rays of dorsal fin.
ec'eth. (*dx.*, *s.*)	Ect-ethmoid or prefrontal (right, left).
eth.	Ethmoid.
eth-f.	Diagrammatic representation of the pseudomesial bar formed by the union of ect-ethmoid and prefrontal.
for. olf. (*dx.*, *s.*)	Foramen for olfactory nerve (right, left).
fv. olf. (*dx.*, *s.*)	Olfactory pit (right, left).
gl. pin.	Pineal gland.
gn. hab.	Ganglion habenula.
ham. eth.	Ethmoid hook in mid-line over mesial cartilage arch.
hy-md.	Hyomandibular.
i'cis. eth. (*dx.*, *s.*)	Ethmoid notch (right, left).
lmn.	Lemniscus (fillet).
lob. olf.	Olfactory lobe.
lob. opt. (*dx.*, *s.*)	Optic lobe (right, left).
mb. ach.	Arachnoid membrane.
ms'eth.	Mesethmoid.
nid. ctx.	Nidulus corticalis (Fritsch).
nid. rot.	Nidulus rotundus.
n. opt. (*dx.*, *s.*)	Optic nerve, right, left).
ob. inf.	See *obl. inf.*
obl. inf. (*dx.*, *s.*)	Inferior oblique muscle (right, left).
obl. su.	Superior oblique muscle.
ob. sv.	See *obl su.*
oc. mig.	Migrating eye.
p.	Posterior.
pall.	Pallium.
pa'sph.	Parasphenoid.
pia	Pia mater.
pin. an.	Anal or ventral fin.
pin. d.	Dorsal fin.
pin. plv.	Pelvic fin.

pt-pal. (*dx.*, *s.*)	Pterygo-palatine cartilage (right, left).
rec. orb.	Recessus orbitalis.
rt. a.	Anterior rectus muscle.
rt. d.	Dorsal rectus.
rt. p.	Posterior rectus.
rt. v.	Ventral rectus.
sul. tct. opt.	Sulcus of *tct. opt.*
tct. opt. (*dx.*, *s.*)	Optic tectum (right, left).
tct. opt. 1	Outer layer.
2	Optic fibre layer.
3	Optic fibrillar layer.
4	Granular layer.
5	Fillet, longitudinal and cross layers.
6	Gray layer.
7	Reticulate and ependymal layer.
trb.	Trabeculæ cranii.
trb. su'orb. (*dx.*, *s.*)	Supraorbital bar (right, left).
trb. su'orb. s. a.	Anterior part of left supraorbital bar.
trb. su'orb. s. p.	Posterior part of left supraorbital bar.
trt. opt. (*d.*, *v.*)	Optic tract (dorsal, ventral part).
tu. co'nt. tis.	Connective tissue sheath.
ur'stl.	Urostyle.
vnt. opt.	Optic ventricle.
I, . . . X	First, . . . tenth cranial nerves.
I. (*dx.*, *s.*)	Olfactory nerve (right, left).
II. (*dx.*, *s.*)	Optic nerve (right, left).
II. d.	Dorsal portion of optic tract.
II. v.	Ventral portion of optic tract.
V. opt. su.	Superior ophthalmic branch of nerve V.
V. opt. pfnd.	Deep ophthalmic branch of nerve V.

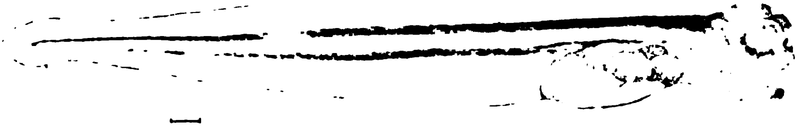
For explanation of Greek letters, see text.

PLATE 1.

(*Pseudopleuronectes americanus.*)

Fig. 1. Recently hatched fish (12 days old) from right side. × 30.
NOTE. — The line indicating the length of this specimen is ½ millimetre too long. The length of the fish was 3.5 millimetres.
Fig. 2. Fish of Stage III. × 10.
Fig. 3. Fish of Stage II. × 10.
Fig. 4. Fish of Stage II, face view. × 35.
Fig. 5. Fish of Stage IV, face view. × 8.
Fig. 6. Fish of Stage IV, from right side. × 8.
Fig. 7. Facial portion of the cartilaginous cranium of a recently hatched fish, Stage I, projected on the frontal plane. × 200.

1



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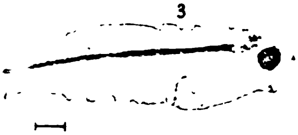

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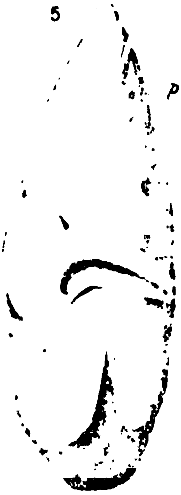

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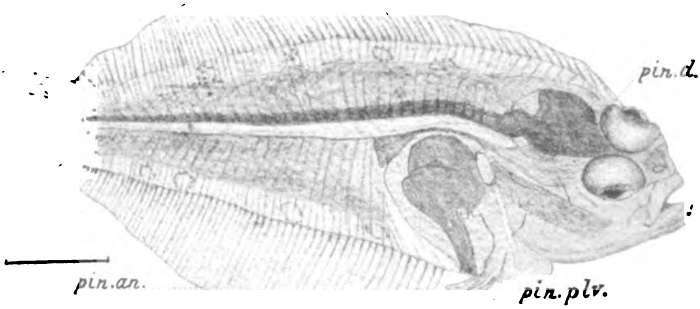


PLATE 2.

(*Pseudopleuronectes americanus.*)

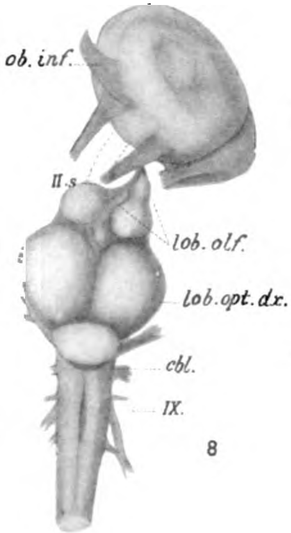
Fig. 8. Brain of fish 75 millimetres long, dorsal view. × 8.
NOTE. — *ob. inf.* should have been *obl. inf.*

Fig. 9. Same brain viewed from right side. × 8.
NOTE. — *ob. sv.* should have been *obl. su.*

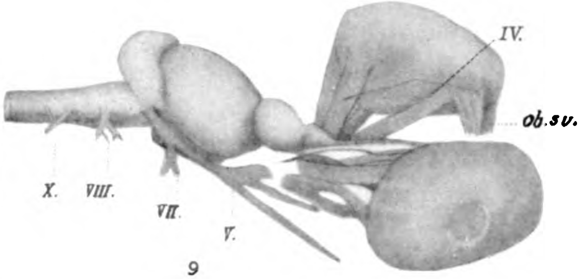
Fig. 10. Facial cartilages of fish of Stage II. as seen from above. × 100.
NOTE — Meckel's cartilage does not extend as far caudad as the lettering, *crt. mk. dx.*, which is placed opposite the quadrate-hyomandibular mass.

Fig. 11. Dorsal view of brain, transposed eye and cranial nerves of adult. From a dissection. × 2.

Fig. 12. Chiasma of a fish at Stage I. seen from in front. × 760.

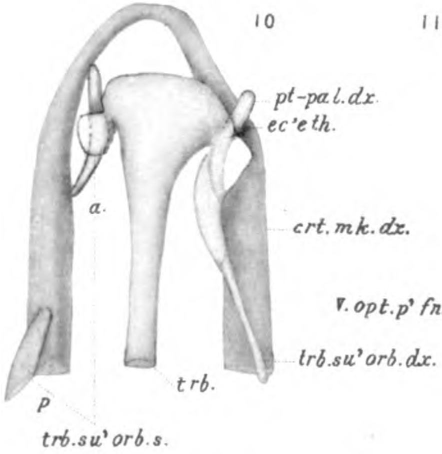

ob. inf.
II. s.
lob. olf.
lob. opt. dx.
cbl.
IX.

8

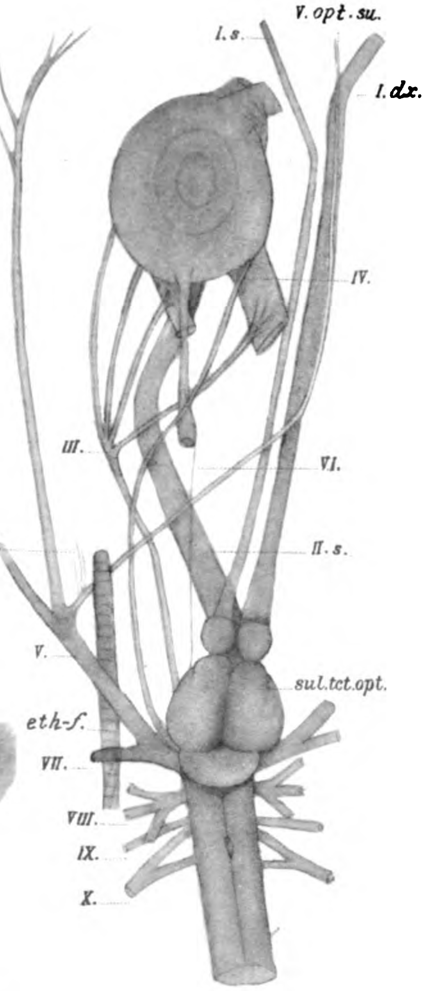

IV.
ob. sv.
X.
VIII.
VII.
V.

9

10


pt-pal. dx.
ec'eth.
a.
crt. mk. dx.
trb. su' orb. dx.
trb.
p
trb. su' orb. s.

11


V. opt. su.
I. s.
I. dx.
IV.
III.
VI.
V. opt. p' fnd.
II. s.
V.
sul. tct. opt.
eth-f.
VII.
VIII.
IX.
X.

12

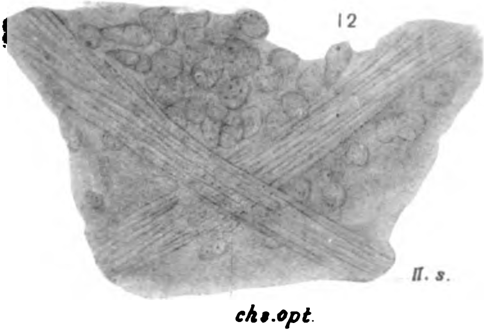

II. s.
chs. opt.

PLATE 3.

Fig. 13. *Bothus*. Anterior face of a cross-section through the nasal pits of a fish in Stage IV. × 40.

Fig. 14. *Bothus*. Dorsal aspect of a frontal section through a fish of Stage II. × 100.

Fig. 15. *Pseudopleuronectes*. Supraorbital bar cut in frontal section showing signs of resorption. × 760.

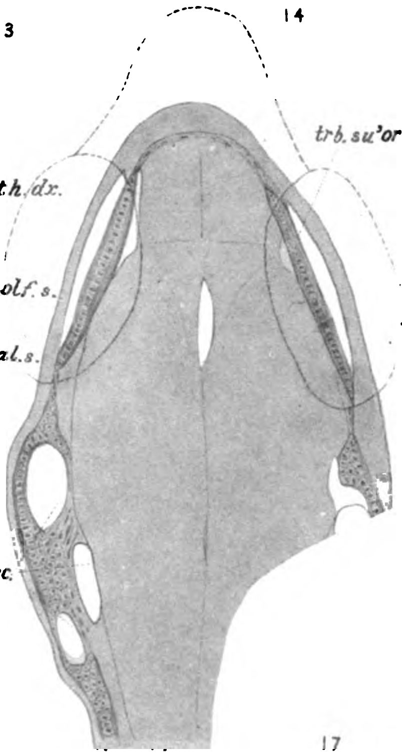
Fig. 16. *Bothus*. Anterior face of a cross-section from the same individual as in Fig. 13. × 40.

Fig. 17. *Pseudopleuronectes*. Anterior face of a cross-section of the head of a fish in Stage I. × 200.

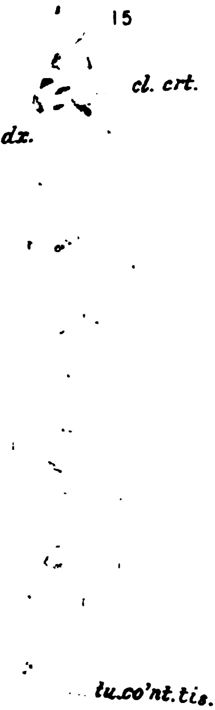
13

crt.pin.d.
pa'sph.

14

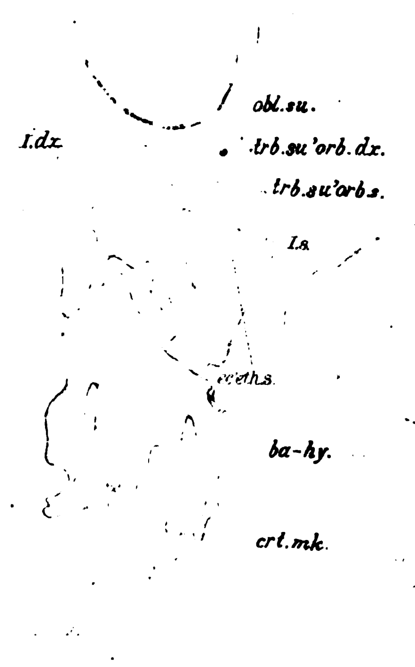

trb.su'orb.dx.
ec'eth.dx.
fv.olf.s.
pt-pal.s.
crt.mk.
can.smi-crc.

15


cl. crt.
tu.co'nt.tis.

16

crt.pin.d.


obl.su.
I.dx.
trb.su'orb.dx.
trb.su'orb.s.
I.s.
ec'eth.s.
ba-hy.
crt.mk.

17

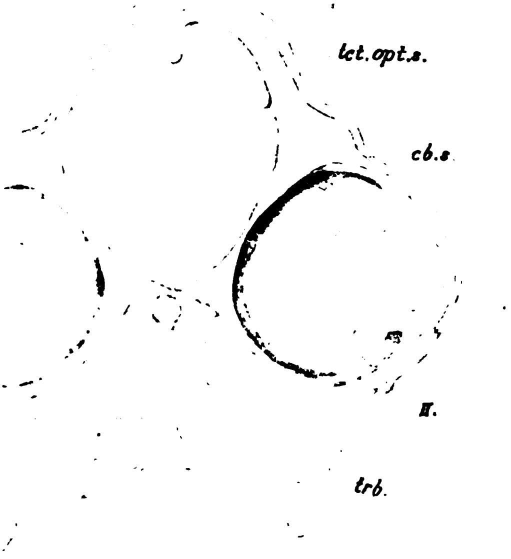

tct.opt.s.
cb.s.
II.
trb.

PLATE 4.

(*Pseudopleuronectes americanus.*)

Fig. 18. Anterior face of a cross section through the head of a fish of Stage III. × 100.

NOTE. — *ob. inf. s.* should have been *obl. inf. s.*

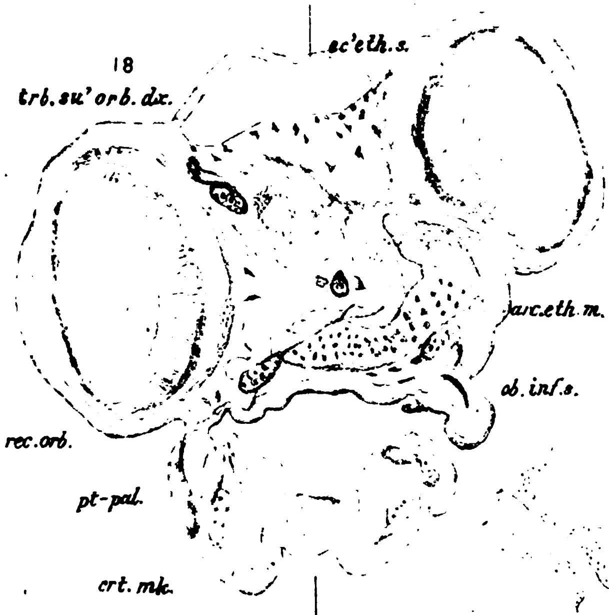
Fig. 19. Portion of a slanting cross section through cerebral lobes and diencephalon. × 100.

NOTE. —The letters *n. opt. s.* in this figure should be changed to *tr. opt. dx.*

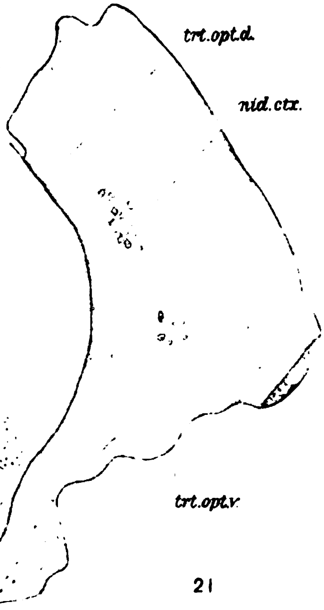
Fig. 20. Frontal section through habenulæ and geniculate bodies. × 100.

Fig. 21. Parasagittal section through geniculate body and optic tract. × 100.

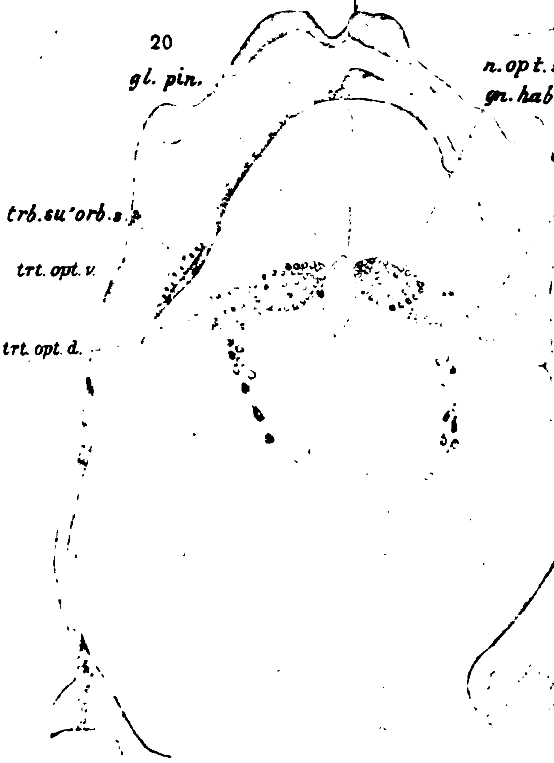
18


ec'eth.s.
trb.su'orb.dx.
arc.eth.m.
ob.inf.s.
rec.orb.
pt-pal.
crt.mk.

19


trt.opt.d.
nid.ctx.
trt.opt.v.

20


gl. pin.
n.opt.s.
gn.hab.
trb.su'orb.s.
trt. opt. v.
trt. opt. d.

21

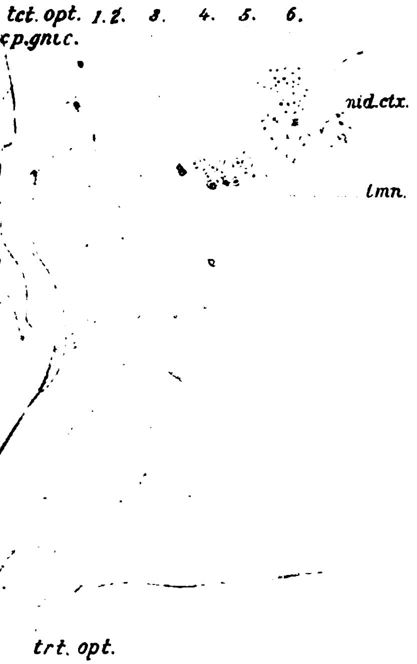

tct. opt. 1. 2. 3. 4. 5. 6.
cp.gnic.
nid.ctx.
lmn.
trt. opt.

PLATE 5.

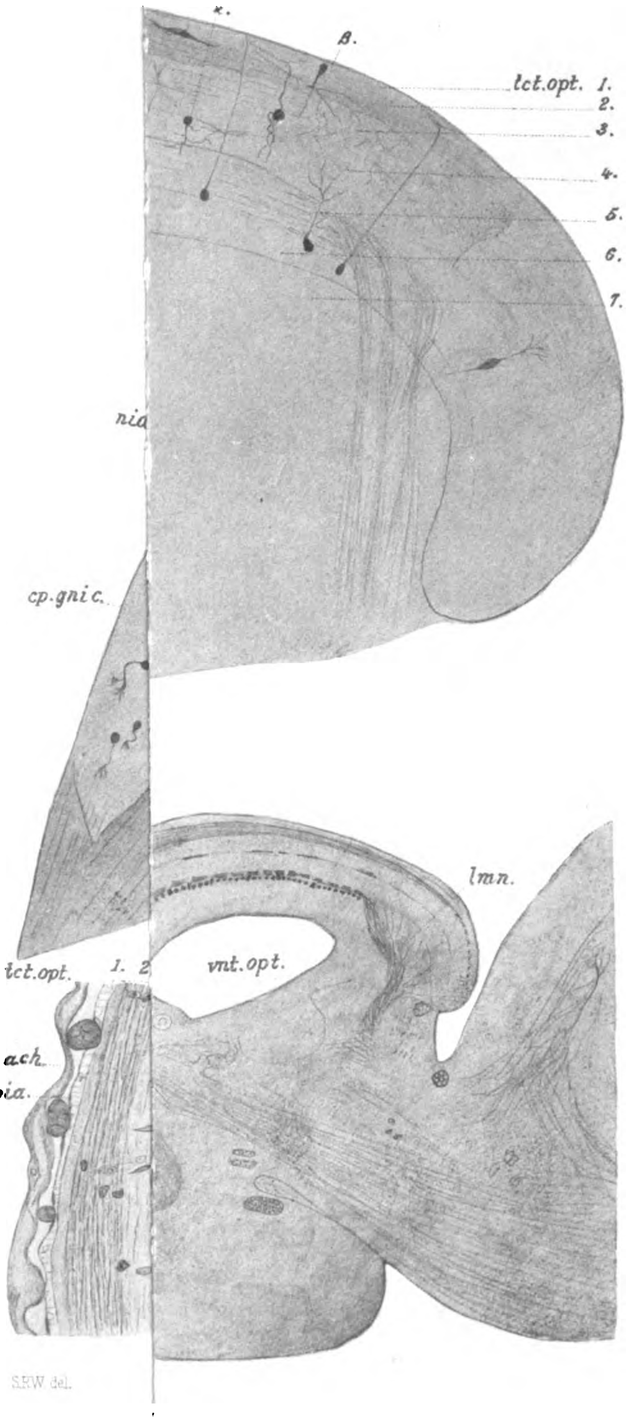
(*Pseudopleuronectes americanus.*)

Fig. 22. Diagram of parasagittal section of tectum. × 57.

Fig. 23. Portion of a parasagittal section of tectum from the anterior part of the optic ventricle to the surface. × 223.

Fig. 24. Cross section of optic nerve. × 50.

Fig. 25. Parasagittal section of diencephalon and part of metencephalon. × 18.



S.R.W. del.

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E. L. Mark, *Director*.

⁂ Abbreviations used:—

B. M. C. Z. . . . . for Bull. Mus. Comp. Zoöl.
P. A. A. . . . . for Proceed. Amer. Acad. Arts and Sci.
P. B. S. N. H. . . . . for Proceed. Bost. Soc. Nat. Hist.

1. Barnes, W.—On the Development of the Posterior Fissure of the Spinal Cord, and the Reduction of the Central Canal, in the Pig. P. A. A. **19**: 97–110. 3 pls. 1884.
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45. Miller, Gerrit S., Jr. — On the Introitus Vaginæ of certain Muridæ. P. B. S. N. H. **26**: 459–468. 1 pl. Feb., 1895.
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47. Wilcox, E. V. — Spermatogenesis of Caloptenus femur-rubrum and Cicada tibicen. B. M. C. Z. **27**: 1–32. 5 pls. May, 1895.
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